ASM 2018 Queensland

Conference Booklet

Welcome from LOC

On behalf of the organising committee and the ASM Executive, it is my pleasure to welcome you to Brisbane for the Australian Society for Microbiology's Annual Scientific Meeting and trade exhibition (ASM2018) at the Brisbane Convention and Entertainment Centre (BCEC). We are proud to present a fantastic scientific and social program with a range of leading scientific speakers from Australia and around the globe.

Microorganisms affect every aspect of life on Earth, and our speakers will cover a wide range of topics that reflect the diversity of microbiology and that highlight the relevance of microbiology to all areas of our daily lives. Our speakers are also from a range of career stages, including Students, Early/Mid-Career Researchers, and Senior Researchers, and we have tried to give as many people the chance to present their work as possible. I hope you all learn a great deal and have the opportunity to catch up with old friends and colleagues, and also have time to make new friends.

I would like to thank all our sponsors for their generous support of the ASM, and I encourage you all to take the time to visit their exhibits and see what they have on offer. Their support is essential and allows us to deliver a meeting of such high standard. There will be plenty of time to interact with the sponsors during the conference, especially during our evening poster, trade and social functions. The Sunday night Welcome Reception and Poster & Trade Session, and the Monday night Poster & Trade Session will be held in the open BCEC Plaza Auditorium Foyer, with canapes/bowl food and drinks on offer. The Tuesday night Rubbo celebration will be held in the BCEC Sky Room that overlooks South Bank and the Brisbane wheel, and will include various food stations, drinks and desert, and even some music and dancing for those interested. During your visit to Brisbane, I also hope you have some extra time to explore surrounding areas. The conference venue is just a few steps from the beautiful Brisbane River and a few minutes from the vibrant city centre, and in close proximity to a host of world-class restaurants, museums (including the Queensland Gallery of Modern Art), and weekend Markets.

Finally, this meeting would not have been possible without many hours of dedication from my colleagues on the organising committee and I would like to thank them all for their hard work - please see the next page for everyone involved. Similarly, the ASM Executive (Roy Robins-Browne, Dena Lyras, Cheryl Power and Jack Wang) have provided invaluable support. I would also like to thank ASN Events (especially Kara Taglieri the ASM National Office Manager) for their work in organising this conference.

I hope that this year's meeting is a rewarding and memorable experience and that we will see you again next year in Adelaide for ASM 2019, June 30 - July 3.

Kate L. Seib
Local Organising Committee Chair, ASM2018

Table of Contents

Section 1 – Introduction and Delegate Information	
Page 5	LOC Members
Page 6	Venue Floor Plan
Page 8	Scientific Chairs Welcome
Page 9	ASM President Welcome
Page 10	National Council
Page 12	Plenary Speakers
Page 16	ASM Awards
Page 18	Delegate Information
Page 20	Social Functions

Section 2 –Scientific Program	
Page 21	Sunday 1st July 2018
Page 22	Monday 2nd July 2018
Page 29	Tuesday 3rd July 2018
Page 37	Wednesday 4th July 2018
Page 44	ASM Special Interest Group Workshops & Meetings
Page 46	Exhibition Floor Plan
Page 50	Sponsor, Supporter & Exhibitor Listing
Page 56	Poster Session 1
Page 59	Poster Session 2

Section 3 – Author Index	
Page 66	Author Index

Section 4 – Abstracts	
Page 76	Oral Abstracts Numbers 1 - 168
Page 136	Author Index Numbers 201 - 404

Section 5 – Delegate List	
Page 198	Oral Abstracts Numbers 1 - 168

LOC Members

LOC Chair

Kate Seib

Scientific Program Chairs

Nick West
Adam Taylor

Committee Members

Amy Jennison (Sponsorship)
Jacqueline Harper (Workshops)
Christopher Day (Abstract coordinator)
Aimee Tan (Social coordinator)
Makrina Totsika
Freda Jen
Alvin Lo
Manisha Pandey
Ian Peak
Erin Shanahan
Tsitsi Diana Mubaiwa (Student/ECR Rep)

Abstract Committee

Kate Seib
Christopher Day
Adam Taylor
Nick West
Amy Jennison
Jacqueline Harper
Aimee Tan
Makrina Totsika
Freda Jen
Alvin Lo
Manisha Pandey
Ian Peak
Evgeny Semchenko
Lara Herrero
Pat Blackall

Venue Floor Plan

Scientific Chairs Welcome

Dear Colleagues,

Welcome to the Australian Society for Microbiology's Annual Scientific Meeting. We are very excited that you can join with us in Brisbane to participate in what will undoubtedly be a truly rewarding event.

We are proud to offer a scientific program, compiled to reflect the broad disciplines represented within our society and to provide a platform towards the advancement of each, while keeping the topics relevant to our members and adhering to the societies principled of equality. The program has been designed to both celebrate the advances made and the challenges ahead, in and for, our central themes of Environmental Microbiology, Clinical Microbiology and Microbial Pathogenesis. Our goal was to bring together global leaders in microbiology research to share knowledge and stimulate discussions while offering continuity across and within disciplines. This is exemplified by such upcoming presentations relating to microbiome research of clinical, environmental and agricultural relevance. Genomics is also represented across the environmental, pathogenesis and health themes. Furthermore we have endeavoured to integrate within themes a vertical progression, such as with One Health, Wildlife and Agriculture Microbiology and Food Safety; additionally the enormous threat of antimicrobial resistance will be examined and backed up by sessions on AMR solutions as well as advances in vaccination.

We are excited to host our internationally eminent orators and plenary speakers and to have them share their expert perspectives in areas of Health Economics (Nicholas Graves – QUT), HIV vaccines (Dennis Burton – Scripps Research Institute), bacterial biofilms (Fitnat Yildiz – UCSC), cost effective microbial diagnoses (Susan Sharp - Kaiser Permanente), bacterial pathogenesis (Michael Jennings – Griffith University), immunological insights to staphylococcal infection (Victor Torres – NYU), diversity of archaea (Anja Spang - Netherlands Institute for Sea Research) and medical mycology (Karl Kuchler - Medical University Vienna). Our Rubbo Orator for 2018 is Paul Young, Professor of Virology and Head of School of Chemistry and Molecular Biosciences at the University of Queensland. Paul brings a wealth of expertise in the field of flavivirus research, notably towards vaccinology and understanding virulence mechanisms of dengue. I encourage you to read the speakers Bio's and session abstracts in the following pages.

Together, symposia speakers, proffered papers, posters, Special Interest Group and specific discipline meetings will provide exceptional opportunities to advance the science of microbiology in Australia and provide an environment in which many collaborations can crystallise.

We wish all delegates a very enjoyable, productive and rewarding meeting.

Nick West
Chair, Scientific Program Committee, ASM2018

ASM President Welcome

Dear fellow microbiologists,

Welcome to the ASM's Annual Scientific Meeting, which is the centrepiece of our 2018 calendar.

This meeting, as most before it, has been two years in the planning. I would like to congratulate everyone who compiled the impressive scientific and social programs, and thank them for their tireless work.

ASM conferences bring microbiologists together from all over Australia and the world. For our members, they provide an invaluable opportunity to update our knowledge and be inspired by the latest breakthroughs in microbiology from the people who made them. These conferences also allow us to catch up with colleagues whom you we not have seen for some time.

For those who are not yet ASM members, I encourage you to join. Becoming a member is easier than ever, and provides many benefits, such as the ability to attend relevant local and international conferences at significantly reduced rates and state-based meetings at no cost, as well as an opportunity to receive prizes and awards that recognise your contribution to microbiology.

More information about ASM, including details on how to become a member, can be found at our website: www.theasm.org.au, where you can also apply for membership online. In the meantime, I wish you a most rewarding and enjoyable conference.

Roy Robins-Browne FASM
ASM president

ASM National Office

The Australian Society for Microbiology
bringing Microbiologists together

9/397 Smith Street
Fitzroy VIC 3065
1300 656 423
www.theasm.org.au

National Council

Executive

President
Roy Robins-Browne

Immediate Past President
Jon Iredell

Vice President, Scientific Affairs & President-Elect
Dena Lyras

Vice President, Corporate Affairs
Cheryl Power

Vice President, Scientific Affairs-Elect
Kate Seib

Vice President, Communications
Jack Wang

Vice President, Communications-Elect
Rebecca LeBard

Standing Committees

National Scientific Advisory Committee
Chair, Prof Dena Lyras

Members:
Linda Blackall
Heidi Drummer
Tom Reilly
Mark Schembri
Deb Williamson
Nick West
Adam Taylor

National Examinations & Qualifications Board
Chair, Prof Julian Rood

Registrar: Gary Lum
Assistant Registrar: Paul Selleck

Members:
David Smith
Andrew Daley
Karena Waller
Kate Seib
Peter Timms
Louise Hafner
Melissa Brown
Tom Riley
Gilda Tachedjian

ASM Student and Early Career Research (ECR) Engagement Coordinator
Priscilla Johansen

Workforce Standing Committee
Chair, Mr Tony Jennings

Members:
Prof Jon Iredell
Dr John Merlino
A/Prof Silvano Palladino
Mr Paul Southwell
Miss Helen Smith
Ms Sue Lloyd Jones

Microbiology Australia Editors:
Dr Ian Macreadie
Mrs Hayley Macreadie
Mrs Rebekah Clark

Members:
Ross Barnard
Mary Barton
Linda Blackall
Narelle Fegan
Gary Lum
Sam Manna
John Merlino
Wieland Meyer
Chris Owens
William Rawlinson
Roy Robins-Browne
Paul Selleck
Erin Shanahan
David Smith
Helen Smith
Jack Wang

National Council

State Branch Chairs

New South Wales & Australia Capital Territory
Mitchell Brown

Queensland
Ulrike Kappler

South Australia & Northern Territory
Stephen Kidd

Tasmania
Anthony Baker

Victoria
Karena Waller

Western Australia
Charlene Kahler

Convenors Of ASM Special Interest Groups

Antimicrobials
Prof Jon Iredell

Bacteriophage
Dr Jeremy Barr

Bioinformatics
Scott Beatson

CDS Users
Dianne Rafferty

Clinical Parasitology and Tropical Medicine
Brooke Taylor

Clinical Serology and Molecular
Dr Linda Hueston

Cosmetics and Pharmaceuticals
Dr Alan Heritage

Culture Media
Peter Traynor

Education
Dr Karena Waller

Eukaryotic Microbes
Prof Alex Andrianopoulos

Food Microbiology
Dr Ed Fox

History
Diane Lightfoot

Medical Mycology
A/Prof Wieland Myer

Microbial Ecology
A/Prof John Bowman

Molecular Microbiology
Hayley Newton

Mycobacteria
Dr Lisa Shephard

Mycoplasmatales
Prof Steven Djordjevic

Public Health Microbiology
Miss Deborah Williamson

Veterinary Microbiology
Fiona Samson

Virology
Prof Suresh Mahaligam

Women's and Children's Microbiology
Anna Maria Costa

Plenary Speakers

Prof Dennis Burton
The Scripps Research Institute
Bazeley Orator

Dennis Burton is the Chairman, and Professor in the Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, USA. He was recently awarded the James and Jessie Minor Chair in Immunology. He received his B.A. in Chemistry from Oxford University and his Ph.D from Lund University, Sweden in physical biochemistry. He is the Scientific Director of the International AIDS Vaccine Initiative (IAVI) Neutralizing Antibody Consortium and Neutralizing Antibody Center, Director of The Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID) at Scripps, and a member of the Ragon Institute of MGH, MIT and Harvard, Boston, USA. He has held many research grants from the NIH and has published more than 350 papers in scientific journals. He has received numerous awards including the Jenner Fellowship of the Lister Institute and a Fellowship in the American Academy of Microbiology. His research is focused on infectious disease, in particular the interplay of antibodies and highly mutable viruses, notably HIV. He is interested in the potential of broadly neutralizing antibodies to inform vaccine design.

Prof Nicholas Graves
Queensland University of Technology
Public Lecture Speaker

I lead AusHSI, a health services research group supported by competitive grants and government funding. We aim to generate information for those who manage health services so they can improve the performance. Simple but important research that increases the value of the health benefits returned from scarce resources is exciting to me. We try to get clinicians to do their own health services research and we share our findings in journals, by giving talks/seminars and using Twitter.

Prof Mike Jennings
Griffith University

Prof Michael Jennings works in the fields of bacterial genetics, bacterial pathogenesis, vaccine development and glycobiology. His undergraduate and postgraduate degrees (PhD 1990) are from Griffith University, Australia. His post-doctoral training was in the laboratory of Prof Richard Moxon at the University of Oxford (1992-1996), supported by a Beit Memorial Fellowship for Medical Research. In 1997 he took up a faculty position at the University of Queensland. In 2009 he returned to Griffith University to take up the position of Deputy Director and Principal Research Leader at the Institute for Glycomics. He has made major contributions to understanding virulence factor function and regulation in a range of bacterial pathogens, including the discovery of epigenetic regulation systems call phasevarions. His current work focuses on the role of glyco-interactions in infectious disease.

Plenary Speakers

Prof Karl Kuchler
Medical University Vienna

Research focus: Karl Kuchler has a long-standing interest in fungal infection biology, molecular genetics, as well as host immune signaling at their intersections with the molecular mechanisms of antifungal drug resistance. His work discovered a novel TLR7-dependent fungal response pathway in dendritic cells, and the group discovered a molecular mechanism underlying the hyper-inflammatory host response upon fungal infections. Finally, the most work uncovered pivotal roles of fungal HDACs/HATs in controlling antifungal drug resistance, stress response, DNA repair as well as virulence.

Dr Susan Sharp
Kaiser Permanente

Dr. Sharp received her B.S. in Medical Technology and her M.S. in Medical Microbiology & Biochemistry from the University of Nebraska Medical Center in Omaha, Nebraska. She received her Ph.D. in Veterinary Microbiology and Parasitology from the Veterinary School of Medicine, Texas A&M University in College Station, Texas. She then did a 2-year post-doctorate fellowship in Clinical Microbiology in the Department of Pathology, Division of Microbiology, at Hartford Hospital in Hartford, Connecticut. She is currently the Regional Director for Microbiology and Molecular Infectious Disease Laboratories at Kaiser Permanente in Portland, Oregon.

Dr. Sharp has been a clinical microbiologist and a very active member of The American Society for Microbiology (ASM) for 30 years serving on various boards and committees. She is a Diplomat of the American Board of Medical Microbiology (ABMM) and a Fellow in the American Academy of Microbiology. Dr. Sharp served as the Chair of the ASM PSAB Committee on Laboratory Practices from 2007 to 2015, as well as Chair of the Examination Development committee and Vice-Chair of the ABMM during 1999-2015. She was selected as the 2012 bioMerieux Sonnenwirth laureate, ASM's highest award given for leadership in clinical microbiology. Dr. Sharp also currently serves as an Advisor to the CLSI Antimicrobial Susceptibility Testing Sub-Committee and is a member of the Board of Scientific Councilors for the Office of Infectious Diseases of the CDC. Dr. Sharp will serve as President of ASM from July 1, 2016 to June 30, 2017.

Dr. Sharp has given numerous lectures, seminars and workshops locally, nationally and internationally, and has numerous publications in the field of clinical microbiology. Her most prominent area of interest has centered on cost-effective, clinically-relevant diagnostic microbiology.

Plenary Speakers

Dr Anja Spang
Royal Netherlands Institute for Sea Research

I did my PhD studies in microbial comparative genomics at the University of Vienna (Austria) and joined the research team of Thijs Ettema at Uppsala University (Sweden) for my post-doctoral research in 2013. During this time, I investigated novel archaeal lineages, that appeared to be key for our understanding of eukaryogenesis. Since September 2017, I work as a tenure track scientist at the Netherlands Institute for Sea Research (Netherlands) and as a VR-funded researcher at Uppsala University. In the coming years, my research will address fundamental questions on the metabolic diversity, symbiotic interactions and evolution of archaea in little explored oceanic environments.

Prof Paul Young
University of Queensland
Rubbo Orator

Paul Young is Professor of Virology and Head of the School of Chemistry & Molecular Biosciences at the University of Queensland. He is the current Chair of the Virology Division of the International Union of Microbiological Societies and a past President of the Australian Society for Microbiology, Australasian Virology Society and the Asia Pacific Society for Medical Virology. He gained his PhD from the London School of Hygiene & Tropical Medicine in 1986 where he began his research into the dengue viruses. He returned to Australia in 1989 and continued his dengue vaccine development program along with research aimed at understanding the molecular basis of dengue virus induced pathogenesis, improved diagnostics as well as therapeutic and vaccine control strategies for the flaviviruses, dengue, West Nile and Zika viruses. His group is also studying the engagement currently being enacted in the wild between a novel retrovirus, KoRV and its koala host and what this tells us about cancer induction and viral evolution.

Current projects include:
The development of a generic recombinant protein platform technology, Molecular Clamp, that locks viral fusion proteins into their pre-fusion state as the basis of subunit vaccine candidates.

Development of TLR4 antagonists as inhibitors of dengue virus induced pathology, based on our groups discovery that the secreted flavivirus protein NS1 is a TLR4 agonist.

Needle-free, micro-array patch delivery to the skin of a range of both established and novel vaccines (inactivated polio, influenza, measles/rubella, dengue, Zika, Ebola etc) with enhanced potency.

The role that koala retrovirus (KoRV) genetic diversity plays in transmission, genome invasion and disease in wild koala populations.

Plenary Speakers

Assoc. Prof. Victor Torres
New York University School of Medicine

Victor received his B.S. in Industrial Microbiology from University of Puerto Rico-Mayaguez campus and his Ph.D. in Microbiology and Immunology from Vanderbilt University in the laboratory of Dr. Timothy Cover. Victor's current research focuses on understanding the staphylococcal pathways involved in responding to host molecules. In January 2009, Victor began a tenure-track appointment as Associate Professor of Microbiology at New York University School of Medicine.

Prof Fitnat Yildiz
University of California, Santa Cruz

Dr. Yildizis an internationally-recognized leader in biofilms and signal transduction. She discovered the structural and regulatory genes required for biofilm matrix and characterized their architectural roles using super resolution microscopy. She pioneered global studies of biofilm gene regulation and has determined the molecular underpinnings of cdiGMP signal transduction pathways, impacting studies across bacteriology. She also contributes substantially to the community by service and teaching.

The central focus of Dr. Yildiz's research is to understand the molecular mechanisms of pathogen transmission and environmental survival using Vibrio cholerae as a model. As a post-doctoral fellow, Dr. Yildiz ascertained that V. cholerae produces a unique extracellular polysaccharide (VPS) that mediates chlorine resistance and promotes biofilm formation. She identified the genes required for VPS biosynthesis and showed, for the first time that VPS gene products are required for biofilm formation. She was part of the team that developed V. cholerae microarrays and was one of the first investigators to use this technology to characterize biofilm formation and transcriptional network analysis. At UCSC, Dr. Yildiz has developed and maintained a thriving interdisciplinary research program addressing fundamental questions on molecular mechanisms of biofilm formation. She identified biofilm matrix proteins, determined chemical structure of VPS, determined assembly principles, architectural roles, and function of biofilm matrix components. She identified and characterized core transcriptional regulatory networks controlling biofilm formation; key c-di-GMP signaling components controlling motile to biofilm transition, and biofilm matrix production. Fitnat received The Ellison Medical Foundation New Investigator award and her research is funded by NIH grants. She has devoted a significant amount of time to the academic community by serving on review panels for NIH and foundations and is a standing member of the NIH BACP Study Section. She has been teaching the Advanced Bacterial Genetics Course at Cold Spring Harbor since 2011. She is actively involved in the organization of the ASM Biofilms conference; serving as a co-chair twice. She has been a session convener and speaker at ASM annual meetings and giving invited talks at conferences worldwide. Fitnat has taught microbiology, bacterial genetics and pathogenesis courses and mentored many scientists who now have productive careers.

ASM Awards

ASM Frank Fenner Award

"The purpose of this award is to recognise distinguished contributions in any area of Australian research in microbiology by scientists in a formative stage of their career, rather than to reward senior scientists for a lifetime of achievement."

Closing Date for applications: March 31st

ASM Lyn Gilbert Award

"The purpose of this award is to recognise major contributions in any area of diagnostic laboratory microbiology in Australia or internationally by ASM members/ fellows."

Closing Date for applications: 31 March of any year.

ASM David White Excellence in Teaching Award

"Applications are invited for the David White Excellence in Teaching Award to recognise excellence in the teaching of, and/or innovation in the teaching of microbiology in Australia."

Closing Date for applications: 31 March in any year

ASM Jim Pittard Early Career Award

"The purpose of this award is to recognise distinguished contributions in any area of Australian research in microbiology by scientists in early stages of their career."

Closing Date for applications: 31 March of any year

ASM Distinguished Service Award

"The Distinguished Service Award recognises outstanding service of, or contributions by, individuals to the Society."

Closing Date for nominations: 31 March in any year

ASM Honorary Life Membership Award

"Honorary Life status is the highest membership recognition given by the Society and carries with it all the rights and privileges of a Member or Fellow together with exemption from payment of the annual membership subscription."

Closing date for nominations: 31 March in any year

ASM Awards

ASM Teachers' Travel Award

"The aim of this award is to encourage ASM members involved in teaching microbiology at the tertiary level to attend the annual scientific meeting of the Australian Society for Microbiology."

Closing date for applications: 31 March of any year

Millis-Colwell Postgraduate Research Travel Award

"Applications are invited from the Australian Society for Microbiology (ASM Aus) members for the Millis-Colwell Award. This award enables the reciprocal exchange of one member between the Australian Society for Microbiology and the American Society for Microbiology (American ASM) to visit a research laboratory in each country and to attend a National Conference of the other society."

NZMS Postgraduate Research Travel Award

"Applications are invited from Australian Society for Microbiology student members for the ASM – New Zealand Microbiology Society (NZMS) Postgraduate Research Travel Award. This award allows for the reciprocal exchange of one student member each year to visit the National conference of the other society and to visit the research lab of a collaborating researcher in that country."

Closing date for applications: 31 March of any year

ASM Nancy Millis Student Award

This award provides the opportunity for one student member from each ASM State Branch to attend and give an oral presentation at the ASM Annual Scientific Meeting.

All postgraduate microbiology students who have submitted or are intending to submit an abstract for the ASM Annual Scientific Meeting are invited to apply - especially those in the final year of their higher degree program.

Closing date for applications: 31 March of any year

ASM Clinical Microbiology Travel Award

"The aim of this award is to encourage ASM members involved in involved in working in the field of clinical microbiology to attend the annual scientific meeting of the Australian Society for Microbiology. "

Closing date for applications: 31 March of any year

Delegate Information

Websites

www.theasm.org.au
asmmeeting.theasm.org.au

Secretariat

ASN Events Pty Ltd
9/397 Smith Street
Fitzroy VIC 3065
Ph: +61 3 8658 9530
Fax: +61 3 8658 9531

Name Badges

You are required to wear their name tags to all scientific and catered sessions

Smoking Policy

Smoking is not permitted in the venue

Taxis

To order a taxi, please speak with Brisbane Convention and Exhibition Centre information desk

Catering

Lunch, morning tea and afternoon tea are included in your registration. Refreshments will be served in the Exhibition Area.

Special Dietary Requirements

If you requested a special meal, please make yourself known to the venue staff and advise your name and special request.

Insurance

The hosts and organisers are not responsible for personal accidents, any travel costs, or the loss of private property and will not be liable for any claims. Delegates requiring insurance should make their own arrangements.

Venue

Brisbane Convention & Exhibition Centre
Merivale St & Glenelg Street
South Brisbane QLD 4101, Australia
Ph: +61 7 3308 3000

Organiser's Registration Desk

The desk will be attended at all times during the conference, see hours below. Delegates should collect their satchel and name tag on arrival. A message board will be at the registration desk to help identify ASM2018.

Sunday 1st July	1:30PM – 7:00PM
Monday 2nd July	7:15AM – 7:30PM
Tuesday 3rd July	8:30AM - 7:00PM
Wednesday 4th July	8:00AM – 2:30PM

Mobile Phones

Please ensure your mobile phone/pager is turned off/ or on silent during any session you attend.

Official Conference Mobile App

The official ASM2018 mobile app will keep you informed during the meeting. Within the mobile app you can view the entire program, plan your own schedule, view your registration, find abstracts and authors by name and make notes for later reference.

Downloading the ASM2018 Mobile App is easy! Simply, visit **asmicro-2018.m.asnevents.com.au** and save the page to your Smartphone's home screen.

Delegate Information

Social Media Guidelines

During ASM's annual scientific meeting and conferences, many of our speakers will be presenting exciting novel research that is not yet published. While the society has an active social media presence, we respect the speakers' right to request that their work not be shared across social media.

The sharing of data without the speakers' consent on publicly accessible platforms may prevent its subsequent publication in scientific journals and compromise their scientific progress. We encourage all attendees to interact on social media by:

- "Liking" ASM on Facebook and sharing your conference experience: facebook.com/AustralianSocietyForMicrobiology
- Following ASM on Twitter: @AUSSOCMIC, and tweeting about the meeting using #2018ASM
- Joining the ASM LinkedIn group: linkedin.com/groups/Australian-Society-Microbiology-6605071
- Subscribing to the ASM YouTube channel: youtube.com/user/AUSSOCMIC
- All talks are "bloggable" and "tweetable" by default, but speakers can explicitly request that certain talks, slides, or findings be left out of the social media conversation. The session chairs will provide clear instructions at the beginning of each talk to highlight any such speaker requests.
-

We ask all attendees to refrain from:

- Recording or reproducing audio, video, or photos of any content presented at oral or poster sessions within ASM scientific meetings. Collecting or distributing this content without the presenter's permission is strictly prohibited.
- The use of rude and profane language to engage in slander or personal attacks across social media platforms.

Oral Presentations

The speaker preparation room is located in the Concord Speakers Prep of the Brisbane Convention Centre. As the program is running concurrent sessions it is essential that you load your talk to the conference network from the speaker prep room at least 2 hours before your session start time. There will be no provision for people to use their own laptops. The conference presentation software is MS PowerPoint 2007. Those preparing on Macs should save for this output. Any issues should be resolved in the speaker preparation room beforehand. A technician will be present to assist.

Poster Presentations

Delegates with posters can place their poster by finding the appropriate abstract number on the display panels in the exhibition area. The program provides instructions regarding the days which posters should be presented. During the specific poster discussion sessions, presenters must be present talk discuss with delegates. This is also the time when official judging will occur.

Disclaimer

The hosts, organisers, venue and participating society is not responsible for, or represented by, the opinions expressed by participants in either the sessions or their written abstracts.

Responsibility for the literary and scientific content of abstracts accepted for publication remains with the authors and their sponsoring institutions. Acceptance by the Society for publication does not imply any acceptance by the Society of responsibility.

Social Functions

Sunday July 1st

Welcome function and first poster session in the Exhibition Area from 6:30 - 8:30pm

It all starts here. Time to greet old friends and make new ones over drinks, snacks and some tastes of superb Queensland food. Enjoy checking out the first poster line up, join in conversations and help get the conference off to a friendly start.

Monday July 2nd

Trade function and second poster session in the Exhibition Area from 7:15 - 8:45pm

Time to keep it all rolling. Relax after an intense and exciting day of presentations. Work your way through the booths of our trade partners and the second poster display. Drinks and snacks provided to sustain you before heading home or down to the waterfront.

Tuesday July 3rd

Rubbo Celebration in the Sky Deck from 6:45 - 8:45pm

A conference tradition. A buffet dinner and drinks will be served with background music and mood lighting to add to the atmosphere. Time to relax, maybe even dance, and celebrate the friendships, science and opportunities to share with, and learn from, each other.

AGM

Tuesday July 3rd

Annual general meeting in the Plaza Audition from 12:30 - 2pm

Listen to the reports from Executive members, ask questions, vote on the proposed constitutional changes and hear about future plans. Your society needs you to attend and participate.

Student & ECR Activities

Monday 2nd July

Nancy Millis Student Breakfast in Meeting Room 9 from 7:30am - 8:45am

Well worth getting out of bed a little earlier for. Sit on a table with a plenary speaker and your fellow students and ask about what possibilities are out there in the world of research and work. One of the most useful and worthwhile breakfasts you will ever have.

Monday 2nd July

Nancy Millis Student and ECR lunch in Meeting Room 9 from 12:30 - 2pm

Pile up a plate of food, then come along to munch away and hear answers to questions you ask yourself or would have liked to have asked. This is a question and answer style format featuring a panel of award winning microbiologists who are making a difference in the field and are happy to share their stories of success.

Scientific Program Sunday 1st July 2018

10:30AM - 1:30PM	**ASM Council Meeting** Invite only.	Concord Boardroom
10:30AM - 2:30PM	**Social Media Workshop** Invite only.	Meeting Room P6
3:00PM - 4:00PM	**Public Lecture - Nicholas Graves** Chairs: Kate Seib & Nick West I will use this lecture to define the discipline of economics and say why it is useful. I will review methods for measuring economic outcomes relevant to those working to prevent infection. I will next discuss how decision makers should use these estimates of economic outcomes to help choose the best among competing programmes. I will finish by arguing that good economics can improve the amount of health gained from an infection prevention service with scarce resources.	Plaza Auditorium
4:30PM - 5:00PM	**Welcome and Awards Ceremony**	Plaza Auditorium
5:00PM - 5:45PM	**Bazeley Oration** Chair: Damian Purcell Session sponsored by CSL	Plaza Auditorium
5:00 PM	**Dennis Burton** Progress toward a neutralizing antibody-based HIV vaccine	abs# 1
5:45PM - 6:30PM	**Plenary 1** Chair: Mark Schembri	Plaza Auditorium
5:45 PM	**Fitnat Yildiz** Mechanisms and regulation of biofilm formation	abs# 2
6:30PM - 8:30PM	**Welcome Function & Poster Session I** Posters #201-#252 on display	Exhibition Area

Scientific Program
Monday 2nd July 2018

7:30AM - 8:45AM	**Nancy Millis Mentoring Breakfast**	Meeting Room P9
9:00AM - 10:30AM	**Public Health - One Health** Chair: Ruiting Lan	Plaza Auditorium
9:00 AM	**Daniel R Knight** Clostridium difficile infection in Australia: not so nosocomial anymore	abs# 3
9:20 AM	**Archie Clements** The epidemiology of Clostridium difficile infections	abs# 4
9:40 AM	**Simon Reid** Getting One Health into practice in Fiji	abs# 5
10:00 AM	**Barbara Drigo** Towards a one health approach: dissemination of antibiotic resistant microbial communities and genes from hospital, municipal wastewater to downstream environments	abs# 6
10:10 AM	**Paraic O Cuiv** The gut microbiota shapes mucosal inflammatory tone and homeostasis in inflammatory bowel disease	abs# 7
10:20 AM	**Mohammad Katouli** Virulence characteristics of adherent-invasive Escherichia coli isolated from healthy individuals, patients and the environment	abs# 8
9:00AM - 10:30AM	**Pathogenesis & Regulation** Chair: Mark Schembri & Freda Jen	Meeting Room P6
9:00 AM	**Victor Torres** Targeting the middle man: Staphylococcus aureus targets human dendritic cells thereby hindering the activation of effector T lymphocytes	abs# 9
9:20 AM	**Yogitha N Srikhanta** Spore Wars: strategies combating spore-forming pathogens	abs# 10
9:40 AM	**Begoña Heras** Structural insights into the regulation and inhibition of bacterial autotransporter proteins	abs# 11
10:00 AM	**Mark A Schembri** Genome-wide discovery of genes required for capsule production by uropathogenic Escherichia coli	abs# 12

Scientific Program
Monday 2nd July 2018

10:10 AM	**Ambika Murthy** Complexity of interactions between uropathogenic E. coli strains and the innate immune system: role of the hemolysin A toxin and the cof phosphatase in initiating human macrophage cell death	abs# 13
10:20 AM	**Marianne N Mégroz** Identification of Pasteurella multocida small RNAs: unravelling the regulatory network	abs# 14
9:00AM - 10:30AM	**Marine Microbiology** Chairs: Philip Hugenholtz & Parisa Noorian	Meeting Room P7
9:00 AM	**Anja Spang** The metabolic potential of ASGARD archaea in light of eukaryogenesis	abs# 15
9:20 AM	**Justin Seymour** After the storm: Microbial community dynamics and antibiotic resistance within an anthropogenically impacted urban beach	abs# 16
9:40 AM	**Nicole Webster** Not available at time of printing	abs# 17
10:00 AM	**Diane McDougald** Vibrio cholerae senses and responds to the presence of the predator, Tetrahymena pyriformis by increasing biofilm formation	abs# 18
10:10 AM	**Erandi Pathirana** Tissue preparation and nucleic acid purification influences the outcome of Pacific oyster (Crassostrea gigas) microbiome studies	abs# 19
10:20 AM	**Nijoy John** Confirmation of anatoxin-a producing cyanobacteria in Australia: evidence of a changing global distribution?	abs# 20

Scientific Program
Monday 2nd July 2018

9:00AM - 10:30AM	**Imaging in Microbiology** Chairs: Glen Ulett & Cynthia Whitchurch	Meeting Room P8
9:00 AM	**Natalie J Spillman** Targeting membrane transporters of the malaria parasite with antitubercular drugs	abs# 21
9:20 AM	**Cynthia Whitchurch** Not available at time of printing	abs# 22
9:40 AM	**Gregory Moseley** Not available at time of printing	abs# 23
10:00 AM	**Matthew J Sullivan** Green Fluorescent Protein as a tool for studying Group B Streptococcal microbiology	abs# 24
10:10 AM	**Iain D Hay** Nanoscale imaging of protein secretion systems used by bacteria and their viruses	abs# 25
10:20 AM	**Hafiz M. N. Iqbal** Nanoparticles loaded chitosan-polyethylene oxide constructs to combat antimicrobial resistance: A drive towards better performance	abs# 26
10:30AM - 11:00AM	**Morning Tea**	Exhibition Area
11:00AM - 11:45AM	**Plenary 2** Chair: Deborah Williamson	Plaza Auditorium
11:00 AM	**Susan Sharp** Cost-effective, Clinically-relevant work up of respiratory and wound specimens	abs# 27
11:45AM - 12:30PM	**Plenary 3** Chair: Jon Iredell	Plaza Auditorium
11:45 AM	**Michael Jennings** The Role of Glycan Interactions in the Cell and Host Tropism of Bacterial Pathogens	abs# 28

Scientific Program
Monday 2nd July 2018

10:30AM - 11:00AM	**Lunch**	Exhibition Area
12:30PM - 2:00PM	**Illumina Workshop** Professor Phil Hugenholtz Microba Illuminating the Human Gut Microbiome	Meeting Room P6
12:30PM - 2:00PM	**Bruker Workshop Meeting**	Meeting Room P8
12:30PM - 2:00PM	**Nancy Millis Student/ECR Lunch**	Meeting Room P9
1:00PM - 2:00PM	**Antimicrobial SIG meeting**	Meeting Room P7
1:00PM - 2:00PM	**CDS Users SIG meeting**	Plaza Auditorium
1:45PM - 3:15PM	**ASM Nancy Millis Student Award Symposium** Chair: Cheryl Power	Plaza Auditorium
	Pramod Subedi Elucidating the Scs redox pathway and its role in copper tolerance in Salmonella	abs# 29
	Kenya E Fernandes Cryptococcus and the Swiss Army Knife of Virulence	abs# 30
	Zoe Bartlett Surveying Bacillus cereus sensu lato in Tasmanian dairy environments and dairy products to inform food safety risk assessments.	abs# 31
	Nicole M Bzdyl Folding your way to Greater Pathogenicity; the role of cyclophilins in Burkholderia pseudomallei virulence	abs# 32
	Carrie F Coggon Presence of inhibitory antibodies in patients with Escherichia coli urosepsis	abs# 33
	Erin B. Brazel Overcoming antimicrobial resistance – exploiting zinc intoxication to restore antibiotic efficacy	abs# 34
3:30PM - 4:00PM	**Afternoon Tea**	Exhibition Area

Scientific Program
Monday 2nd July 2018

4:00PM - 5:30PM	**Microbiology of Wildlife and Agriculture** Chairs: Pat Blackall & Jillian Templeton	Plaza Auditorium
4:00 PM	**Rosalind A Gilbert** Pan-genome of the livestock gut-associated Streptococcus bovis/Streptococcus equinus complex	abs# 35
4:20 PM	**Stuart Denman** The rumen microbiome and its functional relationship with the host animal	abs# 36
4:40 PM	**Joanne Meers** Koala retrovirus infection in Queensland and South Australian koala populations	abs# 37
5:00 PM	**Diane Ouwerkerk** Effect on the rumen microbiomes of cattle shifting from grazing extensive pastures to floodplain pastures in the Northern Territory	abs# 38
5:10 PM	**Angela M Chilton** Cyanobacteria in dryland rehabilitation	abs# 39
5:20 PM	**Ruiting Lan** Population and evolutionary dynamics of Shiga-toxin Producing Escherichia coli O157 in an Australian beef herd	abs# 40

Scientific Program
Monday 2nd July 2018

4:00PM - 5:30PM	**Advances in Vaccinology and Therapeutics** Chairs: Makrina Totsika & Carola Venturini	Meeting Room P6
4:00 PM	**Maria Liaskos** Not available at time of printing	abs# 41
4:20 PM	**Jamie Triccas** Tuberculosis: New therapies for an old enemy	abs# 42
4:40 PM	**Evgeny Semchenko** Neisseria gonorrhoeae vaccine development – targeting of minor outer membrane proteins MetQ and NHBA to elicit host immunity	abs# 43
5:00 PM	**David Lizarraga** Reviewing the effects of chlamydial immunizations on the host immune system	abs# 44
5:10 PM	**Christopher A Mullally** Identification of small molecules as novel therapeutic options that suppress virulence in Neisseria gonorrhoeae and increase susceptibility to CAMPs	abs# 45
5:20 PM	**Nicolas Zaragoza** Improving tetanus toxoid vaccine production	abs# 46
4:00PM - 5:30PM	**Tick and Other Vector-Borne Infectious Diseases** Chair: Gregory Moseley & Lara Herrero	Meeting Room P7
4:00 PM	**Stephen C Barker** Ticks in Australia: endemics; exotics; which ticks bite humans?	abs# 47
4:20 PM	**Stephen R Graves** Tick-borne infectious diseases in Australia	abs# 48
4:40 PM	**Peter Irwin** The search for Australia's tick-borne disease causing pathogens	abs# 49
5:00 PM	**Diane McDougald** Protozoan expelled food vacuoles are an unrecognized vector for the transmission of cholera	abs# 50
5:10 PM	**Eloise Stephenson** Serological evidence for the non-human reservoirs of Ross River virus; Australia's most common arbovirus	abs# 51
5:20 PM	**Amber R Paulson** In vivo transcriptome of insect pathogen Yersinia entomophaga MH96	abs# 52

Scientific Program
Monday 2nd July 2018

4:00PM - 5:30PM	**Viral Pathogenesis** Chairs: Paul Young & Adam Taylor Session sponsored by QIMR/AID	Meeting Room P8
4:00 PM	**Joanne Macdonald** Rapid and smart diagnostics for low resource detection of human and animal diseases	abs# 53
4:20 PM	**Johnson Mak** Novel insights on the genomic RNA packaging and assembly of HIV	abs# 54
4:40 PM	**Dorothy Machalek** Not available at time of printing	abs# 55
5:00 PM	**Damian F Purcell** HIV-1 Env trimers eliciting antibody with neutralising and cellular-dependent functions in vaccinated cows	abs# 56
5:10 PM	**David Harrich** A multiple checkpoint HIV-1 inhibitor can block virus replication in vivo	abs# 57
5:20 PM	**Tristan T Wimpenny** The Role of Exosomes During Infection with Bovine Herpesvirus 1	abs# 58
5:30PM - 5:45PM	**Afternoon Break**	Exhibition Area
5:45PM - 7:00PM	**Award Lectures:**	Plaza Auditorium
	Sam Manna – Jim Pittard Award Variation in the capsular polysaccharide locus of Streptococcus pneumoniae isolates from low and middle-income countries in the Asia-Pacific	abs# 59
	Pat Blackall – Lyn Gilbert Award Diversity – The Dilemma and The Joy of Diagnostic Veterinary Bacteriology	abs# 60
	Anton Peleg – Frank Fenner Award Bacterial Drivers of Neutrophil Behaviour During an in vivo Infection	abs# 61
	Makrina Totsika – Frank Fenner Award My journey with E. coli and urinary tract infections: 15 years, 3 continents, 6 universities and lots of fun on the way.	abs# 62
7:15PM - 8:45PM	**Poster Session II** Posters #301-#403 are on display	Exhibition Area

Scientific Program
Tuesday 3rd July 2018

9:00AM - 10:30AM	**Clinical Micro - Diagnostics & Lab Operations** Chairs: Jacqueline Harper & Robert Norton	Plaza Auditorium
9:00 AM	**Susan Sharp** Rapid Testing for Blood Cultures; Microbiology working with ID and Pharmacy to Maximize Patient Outcomes	abs# 63
9:20 AM	**Rod Givney** The machine comes to bacteriology: automation, agility and disruption	abs# 64
9:40 AM	**Flavia Huygens** A Molecular Gram-Stain for Sepsis: The Silent Killer	abs# 65
10:00 AM	**Seweryn Bialasiewicz** New tools for enhanced diagnosis and characterisation in atypical sepsis	abs# 66
10:10 AM	**Mark N Read** Diagnostics and prognostics through machine learning; a tutorial and case study in gut microbiome-based weight-loss prediction	abs# 67
10:20 AM	**Stacey Hong** Phenotypic and genotypic characterisation of ribotype 251 strains of Clostridium difficile causing severe disease in the Australian community	abs# 68

Scientific Program
Tuesday 3rd July 2018

9:00AM - 10:30AM — **Anti-Microbial Resistance** — Meeting Room P6
Chairs: Anton Peleg & Mohammad Hamidian

9:00 AM
Ella Trembizki
Rapid Laboratory responses to ceftriaxone-resistant Neisseria gonorrhoeae; on behalf of the National Neisseria Network, Australia — abs# 69

9:20 AM
Christopher J Harmer
Transposon maker IS26, flagship of the versatile IS6 family of insertion sequences — abs# 70

9:40 AM
Timothy Kidd
Epidemiology, mechanisms and implications of colistin resistance in Klebsiella pneumoniae — abs# 71

10:00 AM
Shakeel Mowlaboccus
Alarming Expansion of Penicillin-resistant Serogroup W Neisseria meningitidis and Identification of a borderline-ceftriaxone-susceptible Strain in Western Australia — abs# 72

10:10 AM
Ella Johnston
OMV-mediated horizontal gene transfer occurs in Gram negative bacteria — abs# 73

10:20 AM
Muhammad Kamruzzaman
A novel TA system provides plasmid stability and antibiotic tolerance in Enterobacteriaceae — abs# 74

Scientific Program
Tuesday 3rd July 2018

9:00AM - 10:30AM — **Animal Pathogens and Potential Zoonosis** — Meeting Room P7
Chairs: Diane Ouwerkerk & Rosalind Gilbert

9:00 AM
Joanne Devlin
Infectious diseases in Australian wildlife – recent studies performed in partnership with wildlife veterinarians — abs# 75

9:20 AM
Conny Turni
Zoonotic bacterial pathogens — abs# 76

9:40 AM
Cheryl Jenkins
Equine chlamydiosis: emerging threat or under-diagnosed disease? — abs# 77

10:00 AM
Lida Omaleki
Fowl Cholera; a modern insight into an old disease — abs# 78

10:10 AM
John Atack
Multiple bacterial veterinary pathogens contain phase-variable regulons; phasevarions — abs# 79

10:20 AM
Pongthorn Pumtang-on
Campylobacter transmission in Australian free-range broiler flocks — abs# 80

9:00AM - 10:30AM — **Novel Approaches to Virus Control** — Meeting Room P8
Chairs: Patrick Reading & Jillian Carr

9:00 AM
Michael P Gantier
Modulation of antiviral responses by low dose-DNA damaging agents — abs# 81

9:20 AM
Gilda Tachedjian
Vaginal Microbiota and HIV Susceptibility — abs# 82

9:40 AM
Andrew van den Hurk
Not available at time of printing — abs# 83

10:00 AM
Adam Taylor
Novel delivery of a live-attenuated chikungunya virus vaccine candidate — abs# 84

10:10 AM
Verna M Monsanto-Hearne
miR-956 suppression delays viral pathogenicity in model Drosophila melanogaster-virus system — abs# 85

10:20 AM
Martha Zakrzewski
Mapping the virome in wild-caught Aedes aegypti from Cairns and Bangkok — abs# 86

Scientific Program
Tuesday 3rd July 2018

10:30AM - 11:00AM	**Morning Tea**	Exhibition Area
10:30AM - 11:30AM	**Vet Micro SIG Meeting**	Meeting Room P7
11:00AM - 11:45AM	**Plenary 4** Chair: Dena Lyras	Plaza Auditorium
11:00 AM	**Victor Torres** Staphylococcus aureus follows a “DARC” path to lethal infections abs# 87	
11:45AM - 12:30PM	**Plenary 5** Chair: Linda Blackall	Plaza Auditorium
11:45 AM	**Anja Spang** New lights on the phylogenetic and metabolic diversity of archaea and their key role in the evolution of life on Earth abs# 88	
12:30PM -2:00PM	**Lunch**	Exhibition Area
12:30PM - 2:00PM	**Annual General Meeting**	Plaza Auditorium
2:00PM - 3:30PM	**Communication Symposium** Chair: Kate Seib	Plaza Auditorium
	Ben Dobson Communicating with the media	
	Chris Davis Communicating with industry	
	Peter Andrews Communicating with government	

Scientific Program
Tuesday 3rd July 2018

2:00PM - 3:30PM	**Education Symposium** Chair: Karena Waller	Meeting Room P6
2:00 PM	**Terry Mulhern** Act like a scientist! Using physical performance in undergraduate science teaching abs# 89	
2:30 PM	**Vincent Wheatley** Flipping the classroom: design, implementation and outcomes abs# 90	
3:00 PM	**Tracey Bretag** Contract cheating in Australian higher education: Results from a nation-wide survey of students and staff abs# 91	
2:00PM - 3:30PM	**ASM History Symposium** Chair: Cheryl Power	Meeting Room P7
2:00 PM	**Frances Morey** Eradication of Contagious Bovine Pleuropneumonia in Australia abs# 92	
2:30 PM	**Pat J Blackall** Veterinary Bacteriology in Queensland – a personal perspective abs# 93	
3:00 PM	**Jenny Davis** Gram Stains to Genomics: Bacterial Identifications in a Public Health Laboratory abs# 94	
3:30PM - 4:00PM	**Afternoon Tea**	Exhibition Area

Scientific Program
Tuesday 3rd July 2018

4:00PM - 5:30PM

Novel Solutions to Drug Resistance — Plaza Auditorium
Chairs: Julian Rood & Rabeb Dhouib
Session sponsored by QUT/IHBI

4:00 PM
Mark Walker
Reversing antibiotic resistance by destabilization of bacterial metal homeostasis abs# 95

4:20 PM
Sacha J Pidot
New drugs from old bugs abs# 96

4:40 PM
Mitali Sarkar-Tyson
Evaluation of novel inhibitors against the macrophage infectivity potentiator in Gram-negative bacteria abs# 97

5:00 PM
Carola Venturini
Rational design for therapeutic use of bacteriophages against pathogenic bacterial clones abs# 98

5:10 PM
Brinda Chandar
Alternatives in tackling enzyme mediated antimicrobial resistance abs# 99

5:20 PM
Nicky Thomas
Bio-responsive gels containing glycoside hydrolase/gentamicin to combat P. aeruginosa biofilms abs# 100

4:00PM - 5:30PM

Clinical Focus - Atypical Respiratory Diseases — Meeting Room P6
Chair: Nick West

4:00 PM
Paul Bartley
Not available at time of printing abs# 101

4:20 PM
Rachel Thomson
Not available at time or printing abs# 102

4:40 PM
Louise Roddam
Not available at time of printing abs# 103

5:00 PM
Erin P. Price
Integrating genomics and transcriptomics to understand Burkholderia pseudomallei evolution in the cystic fibrosis lung abs# 104

5:10 PM
Brian M Forde
Induced repeat expansion: Characterising a novel mechanism for carbapenem resistance in fatal respiratory diphtheria abs# 105

5:20 PM
Rachael Lappan
Identifying microbial factors protective against recurrent acute otitis media abs# 106

Scientific Program
Tuesday 3rd July 2018

4:00PM - 5:30PM

Food Safety — Meeting Room P7
Chairs: Ed Fox & Conny Turni

4:00 PM
Mark S Turner
Harnessing Environmental Biocontrol Lactic Acid Bacteria For Food Safety and Quality abs# 107

4:20 PM
Lesley Duffy
Survival of Campylobacter through poultry processing abs# 108

4:40 PM
Harriet Whiley
Salmonellosis and eggs: closing the feedback loop from research to regulation abs# 109

5:00 PM
Stanley H Chen
Metagenomic profile of the bacterial community structure on poultry carcasses throughout a factory processing line abs# 110

5:10 PM
Michael Mason
The need for speed: Development of a rapid Campylobacter detection system for the Australian chicken industry abs# 111

5:20 PM
Jillian Templeton
Tracking a naturally recombinant Campylobacter by WGS abs# 112

Scientific Program
Tuesday 3rd July 2018

4:00PM - 5:30PM	**Viral Pathogenesis 2** Chairs: Gilda Tachedjian & Martha Zakrzewski	Meeting Room P8
4:00 PM	**Jillian Carr** Not available at time of printing	abs# 113
4:20 PM	**Patrick Reading** Defining host restriction factors that modulate respiratory virus entry and exit from infected cells	abs# 114
4:40 PM	**Michelle Baker** How do bats clear a viral infection?	abs# 115
5:00 PM	**Daniel J Rawle** HIV-1 RT mutations and small molecules inhibit the interaction between RT and eEF1A and highlight its importance in uncoating, reverse transcription and replication	abs# 116
5:10 PM	**Pyrear SH Zhao** Identification and characteristics of the newly emerged subclades of enterovirus D68 associated with severe respiratory/central nervous system infections in Hong Kong	abs# 117
5:20 PM	**Elisa X.Y. Lim** Ross River virus: does persisting virus and/or genome contribute to chronic disease?	abs# 118
5:30PM - 5:45PM	**Afternoon Break**	Exhibition Area
5:45PM - 6:45PM	**Rubbo Oration** Chair: Roy Robins-Browne	Plaza Auditorium
5:45 PM	**Paul Young** A Virologist in Wonderland: through the looking glass	abs# 119
6:45PM - 8:30PM	**Rubbo Celebration**	Sky Room & Sky Terrace

Scientific Program
Wednesday 4th July 2018

8:30AM - 10:00AM	**Genomics Towards Improved Public Health** Chairs: Scott Beatson & Erin Price	Plaza Auditorium
8:30 AM	**Patrick Harris** Not available at time of printing	abs# 120
8:50 AM	**Amy V Jennison** Refining bacterial disease surveillance: harnessing the benefits of whole genome sequencing in a public health microbiology reference laboratory	abs# 121
9:10 AM	**Deborah A Williamson** Food, sex and genomics	abs# 122
9:30 AM	**Kelly L Wyres** Genomic evolution of Klebsiella pneumoniae clones: the good, the bad and the ugly	abs# 123
9:40 AM	**Verlaine J Timms** The real genomic landscape of Legionella pneumophila in Sydney, Australia	abs# 124
9:50 AM	**Lucy L Furfaro** Whole genome analysis of clinical Group B Streptococcus isolates from Western Australia	abs# 125

Scientific Program
Wednesday 4th July 2018

8:30AM - 10:00AM	**Respiratory Tract Microbiology** Chairs: Jamie Triccas & Aimee Tan	Meeting Room P6
8:30 AM	**Robyn Marsh** Generalisability among respiratory microbiota studies: when does one size fit all?	abs# 126
8:50 AM	**Timothy J Wells** Paradoxical Antibody: The mechanisms and treatment of antibody that exacerbates Pseudomonas aeruginosa lung infections	abs# 127
9:10 AM	**Keith Grimwood** Macrolides: friend or foe in respiratory diseases?	abs# 128
9:30 AM	**Ulrike Kappler** Sugar & Spice – insights into the 'diet' of Haemophilus influenzae in the host	abs# 129
9:40 AM	**Alice (Zheng) Xu** Molecular epidemiology of the 2013-2017 pertussis epidemic in Australia	abs# 130
9:50 AM	**Katharina Richter** Translating a topical treatment for chronic upper respiratory tract infections from bench to bedside	abs# 131
8:30AM - 10:00AM	**Microbial Evolution and Genomics** Chairs: Linda Blackall & Christopher Day	Meeting Room P7
8:30 AM	**Philip Hugenholtz** Single cell viral tagging	abs# 132
8:50 AM	**Heroen Verbruggen** Evolutionary genomics of eukaryotic algae and their microbiota	abs# 133
9:10 AM	**Pierre Offre** Not available at time of printing	abs# 134
9:30 AM	**Clare M Smith** Parallel systems genetics: combining TnSeq and genetically diverse mice to understand TB susceptibility	abs# 135
9:40 AM	**Sian Pottenger** Genomic and functional insights of bacteria affiliated with Clostridium Cluster IV of the core human gut microbiota	abs# 136
9:50 AM	**Yaramah Zalucki** Selection for non-optimal codons in secretory signal sequences is not for weaker mRNA secondary structures in Escherichia coli	abs# 137

Scientific Program
Wednesday 4th July 2018

8:30AM - 10:00AM	**Medical Mycology** Chairs: James Fraser & Ana Traven	Meeting Room P8
8:30 AM	**Karl Kuchler** Out of control – Ablation of the C. albicans Histone Chaperone HIR Drives Fungal Hypervirulence	abs# 138
8:50 AM	**Julianne Djordjevic** Virulence-promoting non-protein kinases: Mechanism of action and exploitation as antifungal drug targets	abs# 139
9:10 AM	**Ana Traven** Metabolic control of host-pathogen interactions in fungal infection	abs# 140
9:30 AM	**Kumar Selvarajoo** Transcriptome-wide Statistical Structure in Saccharomyces cerevisiae Biofilm	abs# 141
9:40 AM	**Laszlo Irinyi** Recent progresses in fungal DNA barcoding	abs# 142
9:50 AM	**Brianna Steed** SwItching it up: Purification of fungal SWI/SNF complexes reveals compositional differences from their yeast counterparts	abs# 143
10:00AM - 10:30AM	**Morning Tea**	Exhibition Area

Scientific Program
Wednesday 4th July 2018

10:30AM - 12:00PM

Bacteriophage, Mobile Elements and Secretion Systems — Plaza Auditorium
Chairs: Ruth Hall & Marina Harper

10:30 AM
Karen Weynberg
Engineering bacteriophages to establish a phage therapy platform for biofilm control — abs# 144

10:50 AM
Jeremy Barr
Tripartite symbioses: Bacteriophage-bacteria-epithelial interactions — abs# 145

11:10 AM
Christopher Rodrigues
The New Kid on the Block - A Specialised Secretion System During Bacterial Sporulation — abs# 146

11:30 AM
Julian I Rood
Identification and characterisation of a new family of conjugative plasmids in Clostridium perfringens — abs# 147

11:40 AM
Rhys Dunstan
Genetic characterisation of Salmonella Typhi and a novel flagellatropic bacteriophage during phage burst — abs# 148

11:50 AM
Minh Duy Phan
Genetic Composition and Regulatory Control of IncA/C Plasmid Conjugation — abs# 149

Scientific Program
Wednesday 4th July 2018

10:30AM - 12:00PM

Glycobiology — Meeting Room P6
Chairs: Johanna Kenyon & Lucy Shewell
Session sponsored by Institute for Glycomics / Griffith University

10:30 AM
Fitnat Yildiz
Vibrio cholerae biofilm matrix assembly and function — abs# 150

10:50 AM
Benjamin L Schulz
Folding and Froth: Glycosylation in yeast in protein biosynthesis and sparkling wines — abs# 151

11:10 AM
Jaclyn S Pearson
Salmonella sweet talking it's way around host innate responses — abs# 152

11:30 AM
Christopher Day
Glycan-glycan interactions between host glycans and pathogen glycans: Role in colonisation/adherence — abs# 153

11:40 AM
Vikrant Minhas
Utilisation of the sugar raffinose dictates disease progressionin Streptococcus pneumoniae — abs# 154

11:50 AM
Freda E.-C. Jen
High frequency changes in pilin glycosylation patterns of epidemic serogroup A Neisseria meningitidis strains in the African meningitis belt — abs# 155

Scientific Program
Wednesday 4th July 2018

10:30AM - 12:00PM	**Tropical, Regional and Point of Care Medicine** Chairs: Flavia Huygens & Seweryn Bialasiewicz	Meeting Room P7
10:30 AM	**Gemma Robertson** Not available at time of printing	abs# 156
10:50 AM	**Robert Norton** Q fever in North Queensland - The changing epidemiology	abs# 157
11:10 AM	**Brenda Govan** Can we develop of a diagnostic assay to detect and monitor rheumatic heart disease?	abs# 158
11:30 AM	**Kyra Cottrell** Exploration of the upper respiratory tract microbiota of remote Australian Aboriginal children – implications of season and household occupancy	abs# 159
11:40 AM	**Martina Jelocnik** Diagnosing Chlamydia psittaci and Chlamydia pecorum in less than an hour using rapid novel isothermal amplification assays	abs# 160
11:50 AM	**Fiona M Sansom** The role of nicotinamide adenine dinucleotide (NAD) synthesis / in the pathogenesis of Coxiella burnetii	abs# 161
10:30AM - 12:00PM	**Fungal Ecology and Taxonomy** Chairs: Julianne Djordjevic & Laszlo Irinyi	Meeting Room P8
10:30 AM	**Michael McDonald** Experimental co-evolution of Yeast	abs# 162
10:50 AM	**Joanne H Connolly** Mucormycosis in the platypus and the amphibian	abs# 163
11:10 AM	**Kaylene Bransgrove** Tropical fungal endophytes, mountain islands and host preference	abs# 164
11:30 AM	**Linda Henderson** Copper (II) Lead (II) and Zinc (II) reduce the growth, zoospore production and attachment rate to organic substrates of three species of zoosporic fungi from soils of NSW	abs# 165
11:40 AM	**Edward D Kerr** Fermenting Yeasts and Where to Find Them	abs# 166

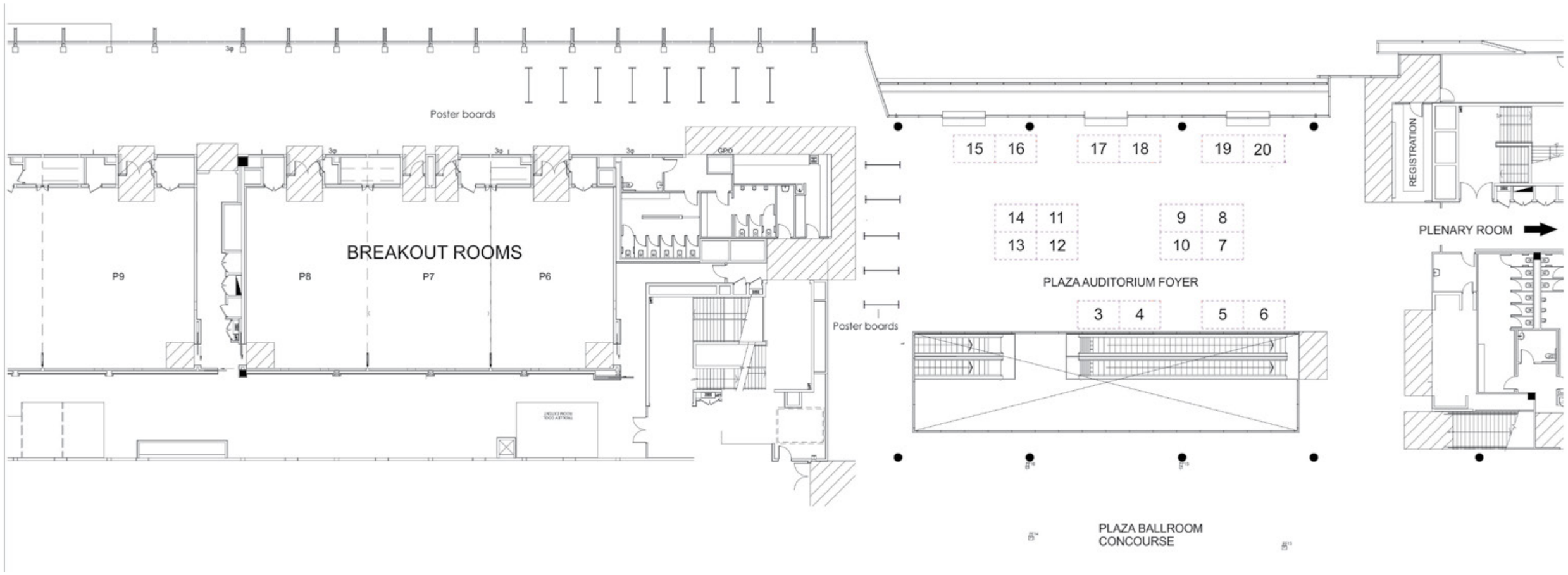
Scientific Program
Wednesday 4th July 2018

11:50 AM	**Stephanie Owen** Identifying changes in painted and polyester surfaces during the attachment and proliferation of three species of environmental fungi using Synchrotron-sourced macro ATR-FTIR microspectroscopy	abs# 167
12:00PM - 12:05PM	**Delegates to move to Plaza Auditorium for Plenary/closing address**	
12:05PM - 12:50PM	**Plenary 6**	Plaza Auditorium
12:05 PM	**Karl Kuchler** Out of control – Ablation of the C. albicans Histone Chaperone HIR Drives Fungal Hypervirulence	abs# 168
12:50PM - 1:00PM	**Closing Address**	Plaza Auditorium
1:00PM - 2:00PM	**Lunch**	Exhibition Area

ASM Special Interest Group Workshops & Meetings

Day/Time	Workshop/Details	Location	Contact
Wednesday 2:00pm-3:30pm	**Culture Media SIG Workshop** Title: ASM Guidelines for the Quality Control and Quality Assurance of Microbiological Media Medical; mycology; solid TB; Food & Water microbiology	Meeting Room P8	Peter Traynor
Wednesday 2:00pm-3:30pm SIG AGM 4:00pm-4:30pm	**Eurkaryotic Microbes SIG Workshop** Title: Cool tools for studying eukaryotic microbes Synopsis: Eukaryotic microbes are fantastic model systems for deciphering the fundamental mechanisms of eukaryotic molecular and cell biology. In addition, fungi and parasites are serious pathogens of humans, animals and plants. The workshop will discuss advanced approaches for studying the biology and pathogenesis of eukaryotic microbes. Speakers: Timothy Tucey (Monash University) Lucia Zacchi (University of Queensland) Brianna Steed (University of Melbourne) Aaron Jex (Walter and Elisa Hall Institute) Ala Tabor (University of Queensland) James Fraser (University of Queensland)	Meeting Room P6	Ana Traven
Wednesday 2:00pm-5:30pm	**Genomics Workshop (Bioinformatics/Public Health & Food Microbiology SIGs)** This workshop presents practical guidelines for interpreting whole genome sequence (WGS) data from bacterial pathogens. We will focus on how WGS can be applied to investigate suspected outbreaks, especially those caused by food-borne disease-causing bacteria. Strategies for using Illumina, PacBio and Nanopore platforms will be introduced along with direct metagenomic sequencing of clinical samples. We will include an interactive session on using freely available cloud-based platforms for undertaking applied bacterial genomics analysis. Although targeted at Food Safety, Public Health or Clinical microbiologists this workshop will be of interest to anyone seeking practical strategies and tools that they can use to investigate their own bacterial WGS data. • • Laptop computer is recommended but not required. • Details of how to access Galaxy cloud server and tutorials will be provided to all registered participants prior to the workshop. • No previous bioinformatic experience required.	Plaza Auditorium	Scott Beatson Ed Fox Deborah Williamson
Wednesday 2:00pm-5:30pm	**Clinical Serology and Molecular Biology Workshop** Title: Old diseases, new challenges in diagnosis Modern medicine has seen a reduction in the prevalence of many infectious diseases, particularly in the last 60 years. Development of improved vaccines, better treatment options and government sponsored vaccination programmes have undoubtedly improved human health. This has led to the belief that many diseases with high morbidity and mortality rates have been eliminated or eradicated. Yet to date, the only human disease declared eradicated is smallpox. We have exerted a measure of control on many of the childhood killers of the past and some are moving towards elimination status – but they haven't gone away. In fact over the last decade many of these "old diseases" are making a comeback. Measles for example killed 2.6 million people worldwide in 1980, by 2000 this was reduced to 550,000 and by 2016 was reduced to 89,780. Despite this obvious improvement we have seen outbreaks in 2015 across multiple states of the US originating from one traveller to Disneyland. Measles outbreaks have spiked in 15 European countries in 2017. Australia has seen several outbreaks in multiple states following introduction by viraemic travellers.	Meeting Room P7	Linda Hueston

Exhibition Floor Plan



Organisation	Booth No.
Australian Society for Microbiology	12
BioMerieux	11
Bruker	8
Cell Biosciences	4
Copan	10
DKSH	18
Gene Target Solutions	13

Organisation	Booth No.
Illumina Australia	20
MP Biomedicals Australasia	17
Ramaciotti Centre For Genomics	19
R-Biophram Australia	5
Sysmex Australia	15-16
Tecan Australia	3
Thermo Fisher Scientific	7
Zeiss	6

Bronze Sponsors

Bruker - Booth 8

Bruker continues to expand on its comprehensive range of innovative mass spectrometry systems. The compact, impact II™, and maXis II™ and new Tims TOF ESI–QTOF mass spectrometers are the showcase instrument platforms for life science research, drug discovery & development, and screening applications involving analysis of unknown compounds in complex matrices. The systems provide cutting edge performance in one-shot analysis for identification and quantitation from small molecules up to antibodies. The rapifleX™ MALDI Tissuetyper™ and, solariX XR ESI/MALDI FTMS both use MALDI IMAGING as a leading Toxicology validation, verification method for pharmaceutical lead drug development projects success.

GSK

GSK – one of the world's leading research-based pharmaceutical and healthcare companies – is committed to improving the quality of human life by enabling people to do more, feel better and live longer.

GSK is one of the world's leading vaccine companies, involved in vaccine research, development and production. We have 14 vaccines in development and our portfolio of 41 vaccines prevent illnesses such as hepatitis A, hepatitis B, diphtheria, tetanus, whooping cough, measles, mumps, rubella, polio, typhoid, influenza and bacterial meningitis. Globally, we have more than 16,000 people working to deliver more than 2 (2.3) million vaccines every day, to people in 166 countries.

Bronze Sponsors

Illumina - Booth 20

Illumina's next-generation sequencing (NGS) takes you inside microbiology. Putting the big picture into focus by revealing the smallest of details.

- Characterise un-culturable organisms
- Develop new strategies to control outbreaks
- Monitor host-pathogen interactions

NGS is opening new doors in microbial genomics, revealing fresh insight into how microbes impact humans and the environment.
Through the power and high resolution of Illumina technology, you can now understand the genetic makeup of organisms that were previously impossible to study – helping you examine microbial biological functions, track genetic changes, monitor food sources and more.

ThermoFisher - Booth 7

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through our Thermo Scientific and Invitrogen brands, we help our customers accelerate and enhance productivity.

Sponsor Listing

Bazeley Oration Sponsor

CSL

CSL is a global specialty biopharmaceutical company that researches, develops, manufactures and markets biotherapies to treat and prevent serious and rare medical conditions. We produce safe and effective therapies for patients who rely on them for their quality of life and sometimes for life itself, enabling many thousands of people around the world to lead normal healthy lives.

CSL employs over 13,000 staff in more than 27 countries. Our headquarters are in Australia and we have substantial manufacturing operations in the US, Germany, Switzerland and Australia. CSL also has one of the largest plasma collections networks in the world and operates the only influenza vaccine manufacturing facility in the Southern Hemisphere.

Rubbo Oration Sponsor

Rubbo Trust

Conference App Sponsor

Thermofisher

Thermo Fisher Scientific is the world leader in serving science. Our mission is to enable our customers to make the world healthier, cleaner and safer. Through our Thermo Scientific and Invitrogen brands, we help our customers accelerate and enhance productivity.

Sponsor Listing

Symposium Sponsors

AID/QIMR

The Australian Infectious Diseases Research Centre (AID) links research at QIMR Berghofer Medical Research Institute and UQ. It includes researchers within the Infectious Diseases Programme at QIMR Berghofer Medical Research Institute, University of Queensland's Faculties of Science and Medicine, The University of Queensland Centre for Clinical Research, UQ Children Health Research Centre, the Institute for Molecular Biosciences, the Australian Institute for Bioengineering and Nanotechnology and the Diamantina Institute. AID combines strong basic and clinical research in infectious diseases with global imperatives.

Griffith University

Griffith University was created to be a different kind of university—challenging conventions, responding to trends and pioneering solutions. Ranking in the top three per cent of universities worldwide, its future-focused degrees are developed in consultation with industry, based on cutting-edge research, and taught by Australia's most awarded teachers.

IHBI/QUT

QUT's Institute of Health and Biomedical Innovation (IHBI) uses the latest technology and brings together thebest minds from around the world to develop solutions for health problems affecting the global community. IHBI has more than 1300 members representing a broad spectrum of disciplines, with a research focus on prevention, intervention and translation.

UQ

The School of Chemistry and Molecular Biosciences (SCMB) at The University of Queensland (UQ) teaches and researches in the disciplines of Microbiology, Parasitology, Chemistry, Biochemistry and Biotechnology. We use molecular-based approaches to understand life, detect and treat diseases, and design and test new pharmaceutical and diagnostics products.

Exhibitor Listing

The Australian Society for Microbiology - Booth 12
www.theasm.org.au
The ASM is a not-for-profit organisation, formed in 1959 as a learned society devoted to furthering the science of microbiology. The ASM became an incorporated professional society in 1976.

bioMerieux - Booth 11
www.biomerieux.com

bioMerieux continues its commitment to improving healthcare worldwide through a complete range of in-vitro diagnostic solutions for managing Infectious diseases, Cardiovascular diseases and targeted Cancers. The acquisition of Biofire has strengthened our leadership position in molecular diagnostics and syndromic testing. Committed to our strategy of addressing the major challenges for public health. bioMerieux innovate and design the diagnostics of the future, whilst improving laboratory efficiency and turnaround time for the benefit of the patient.

Cell BioSciences - Booth 4
www.cellbiosciences.com.au

Cell Biosciences Pty Ltd offers a select range of products, supported by an experienced team.

Biolog – advanced ID and phenotyping systems.
Interscience – scientific equipment for microbiology.
Microbiologics – QC microorganisms and controls. Qualitative and quantitative.
Neogen – Food Safety products. Hygiene, spoilage and pathogen detection.
Pro-Lab Diagnostics – diagnostics and organism storage systems.
Puritan Medical – patented flocked swabs for bacterial, viral and molecular testing.
SwiTrace – temperature loggers to monitor in-transit temperatures.

COPAN - Booth 10
www.copangroup.com

With a reputation for innovation, COPAN is the leading manufacturer of collection and transport systems in the world. COPAN's collaborative approach to innovation in pre-analytics has resulted in the original FLOQSwabs™, ESwab™, FecalSwab™ and UTM™, as well as Full Laboratory Automation. COPAN's collection and systems have been proven to advance the quality of traditional and contemporary microbiology assays. COPAN's automation includes specimen processing, smart incubation, digital imaging, and strong algorithms for automatic segregation of bacterial cultures, followed by automated colony picking.

Exhibitor Listing

DKSH - Booth 18
www.dksh.com

DKSH Scientific Instrumentation operates within Business Unit Technology, and offers a complete portfolio of analytical instruments, general equipment, and consumables to laboratories in government, research institutes, universities, contract analysis and industrial sectors.

We also offer a total care solution for the products we supply. This total care approach encompasses pre-sale, installation, application, warranty, training and after sales support. We provide professional support to help customers solve complex challenges, improve results and increase productivity.

We are a solution provider in these markets:
Academic / Government Research
- Material Science
- Life Science
- Environmental
- Energy Resources
- Food, Diary and Beverage

Genetic Signatures - Booth 14
www.geneticsignatures.com

Genetic Signatures is transforming molecular diagnostics. Unique 3base™ technology improves infectious disease screening, with Flexibility, High Throughput and an Expansive Menu offering with EasyScreenTM kits. EasyScreenTM simplifies molecular diagnostics by specifically and simultaneously screening multiple pathogens in one standardized process. EasyScreenTM kits include Enteric, Respiratory, STI, Anti-Microbial Resistance, Tropical Disease Pathogens and Meningitis.

Gene Target Solutions - Booth 13
www.geneticsignatures.com

Gene Target Solutions is local Australian company which is a Gateway to many exciting Molecular Technologies and Innovations. qPCR/qRT-PCR, are key technologies used in Molecular Diagnostics and yet the data generated on qPCR instruments being used, are often analysed incorrectly. Please visit our booth to gain further insight into an area not well understood.

Exhibitor Listing

MP Biomedical - Booth 17
www.mpbio.com

MP Biomedicals supplies the market-leading FastPrep homogeniser systems which can process up to 96 samples in a single high-powered run. Sample preparation is critical to the quality and yield of your final product. We will advise you on the best lysing matrix, extraction and purification options to ensure your success.

Ramaciotti Centre For Genomics - Booth 19
www.ramaciotti.unsw.edu.au

The Ramaciotti Centre for Genomics is a national infrastructure facility and a focus for the development and application of genomics in Australia.

Our purpose is to deliver internationally competitive genomic services by providing our clients with access to enabling technology and services of the highest quality. The Centre's technology suite includes long- and short-read next-generation sequencing, single-cell analysis, microarrays and capillary sequencing.

R-Biopharm - Booth 5
www.labdiagnostics.com.au

R-Biopharm Australia (Laboratory Diagnostics) is a company dedicated to sourcing and providing innovative niche quality products and solutions from their own corporate product range as well as a number of internationally recognised manufacturers.

We are an emerging PCR company providing a comprehensive range of PCR products for the Medical, Food and Veterinary testing industry developed and manufactured by our parent company based in Germany.

Sysmex - Booth 15-16
www.sysmex.com.au

Sysmex Australia specialises in the delivery and implementation of clinical IVD and health IT products and services for clinical laboratories, hospitals and healthcare organisations. Stop by Booth 15 & 16 to check out how our flexible and modular innovative solutions can help improve your workflow efficiency.

Exhibitor Listing

Tecan - Booth 3
www.tecan.com

Tecan is a leading global provider of automated laboratory instruments and solutions. Our systems, components and support help people working in clinical diagnostics, basic, translational research and drug discovery bring their science to life. We develop, produce, market and support automated workflow solutions that empower laboratories to achieve more. Our expandable, reconfigurable solutions cover a number of microbiology workflow needs including efficient colony picking, nucleic acid purification, PCR set up, spiral plating/streaking, antimicrobial susceptibility testing and more.

Wishmed - Booth 9
www.wishmed.com.au

Wishmed was established in March 2010 as a technical consultancy to support Lab equipment installations and repairs. In Feb 2011, we took the steps to come in lab supply field after finding unregulated priced product in the market. We decided to bring in the quality products from Italy, Germany, France, Taiwan and India at an affordable and same price range all across the board whether it is Diagnostic pathology lab normally paying very low prices or research labs paying very high on the same products.

Wishmed deals in general laboratory consumables, Laboratory Equipment, Medical Consumables, Medical Equipment, Refrigerated and non refrigerated and smart storage systems based on the most innovative RFID technology for laboratories and blood banks.

We share a strategic partnership with all our suppliers. Wishmed provides these quality European products to the vast and vibrant market of Australia New Zealand and Pacific Islands. These products cater to the needs of Diagnostic, Research, food, pharma and Vet laboratories in both Private and Government sectors.

Zeiss - Booth 6
www.zeiss.com.au

Zeiss is a leading international group of companies operating worldwide in the optical and opto-electronic industries. Offering a comprehensive range of microscopy technologies ranging from light, electron, ion and X-ray microscopes, ZEISS is enabling researchers in life sciences, material science, industrial and clinical applications to achieve new scientific discoveries.

Poster Session 1

John R Melki	Evaluation of EasyScreenTM ESBL/CPO Detection Kit using direct-PCR from patient culture and broth samples	abs# 201
Dhruba Acharya	Role of flagella as an immune modulator during urinary tract infection	abs# 202
Julie K Allerton	A Comparison of the Results of Three Methods of MIC Determination	abs# 203
Julie K Allerton	A Review of Polymyxin Disc Testing from a Calibrated Dichotomous Susceptibility Perspective	abs# 204
Charlene Kahler	Genetic diversity and distribution of filamentous phages in Neisseria	abs# 205
Fabian Davamani Amalraj	Establishment of bacterial microbial biofilms from Antarctic soil bacteria	abs# 206
Norvie L Aquino	Quality assurance program for molecular detection of gastrointestinal parasites: results of pilot module	abs# 207
Melinda M Ashcroft	New insights into the functional roles of DNA methylation in extraintestinal pathogenic Escherichia coli	abs# 208
Su Aye	Lipid A profiling and metabolomics of polymyxin-susceptible (PBS) and -resistant (PBR) MDR Klebsiella pneumoniae	abs# 209
Ammar Aziz	Genomic and transcriptomic analysis of nontypeable Haemophilus influenzae from paediatric chronic lung disease patients	abs# 210
Stephanie L Begg	Antimicrobial mechanisms of arachidonic acid at the host-pneumococcal interface	abs# 211
Mahboobeh Behruznia	Identifying persistent E. coli strains to harsh life outside the host	abs# 212
Jan Bell	Antimicrobial use and resistance in australia (aura) surveillance system: recent developments	abs# 213
Jan M Bell	Update on the National Alert System for Critical Antimicrobial Resistances (CARAlert)	abs# 214
Erwin M Berendsen	Introducing Microbial Culture-Metagenome Sequencing (MC-MGS) to characterize gut mucosa-associated microbial communities	abs# 215
Murari Bhandari	Investigating the role of DsbA enzymes in growth and virulence of uropathogenic Escherichia coli	abs# 216
Natalie J Bitto	Staphylococcus aureus membrane vesicles activate innate immune signalling pathways	abs# 217
Pat Blackall	Staphylococcaceae and Pasteurellaceae found in the oral flora of marsupials and dingoes	abs# 218

Poster Session 1

Cameron Buckley	Whole genome sequencing as an improved means of identifying Neisseria gonorrhoeae treatment failures	abs# 219
Sean J Buckley	In silico characterisation of the two-component system regulators of Streptococcus pyogenes	abs# 220
Michael A Burch	Small RNA-mediated regulation in Acinetobacter baumannii	abs# 221
Andrea Bustamante	Molecular epidemiology of Mycobacterium abscessus complex in NSW	abs# 222
Michelle N Chamoun	UPEC Pathway to Chronicity: Bacterial Strain and Host Differences Determine Chronic Outcomes	abs# 223
Deirdre A Collins	Microbiological evaluation of the DEKO-190 Washer/Disinfector's ability to remove Clostridium difficile spores from bedpan surfaces	abs# 224
Corey B. Davies	Pseudomonas aeruginosa Trent and zinc homeostasis	abs# 225
Dewa Rasmika Dewi	Diversity of carbapenem resistance bacteria and molecular identification of carbapenem resistance in marine environment	abs# 226
Divya Dharshini	Response of Antarctic psychrotrophic bacteria to antibiotics before and after exposure to heavy metals	abs# 227
Rabeb Dhouib	Dsb enzymes: promising anti-virulence drug targets for Gram-negative pathogens	abs# 228
Michael P Doane	The major evolutionary split between elasmobranchs and teleost fishes extends to the diversity partitioning of the skin microbiomes	abs# 229
Paulina A Dzianach	Computational Model of Campylobacter jejuni Biofilms	abs# 230
Juhee Ahn	Characterization of bacteriophages with specific for antibiotic-resistant Salmonella Typhimurium	abs# 231
Robert A Edwards	Global phylogeography and ancient evolution of the widespread human gut virus crAssphage	abs# 232
Li Ching Eng	Evaluation of Short Incubation Disc Antimicrobial Susceptibility Testing	abs# 233
Li Ching Eng	Detection and Surveillance of VISA and hVISA strains	abs# 234
Rininta Firdaus	Meningococcal PorA-Loop4 induces G1 cell cycle arrest through the Akt signaling pathway	abs# 235
Robert L Flower	Variation in parvovirus B19 IgG seroprevalence between different states in Australia	abs# 236

Poster Session 1

Winkie Fong	Comparison of pre-extraction depletion methods for culture-independent sequencing of Bordetella pertussis	abs# 237
Edward M Fox	Food loss – the microbiology of food deterioration	abs# 238
Lucy L Furfaro	Group B Streptococcus bacteriophages with broad clinical host range	abs# 239
Stephen R. Graves	A preliminary comparison of five assays for detecting past exposure to Coxiella burnetii for use prior to human Q fever vaccination	abs# 240
Jessica Gray	Biofilm screening of Listeria monocytogenes from common MLST subtypes present in food production environments.	abs# 241
Emily L Gulliver	The role of the RNA chaperone ProQ in the Gram-negative bacterium Pasteurella multocida.	abs# 242
Mohammad Hamidian	Evolution of a Clade of Acinetobacter baumannii Global Clone 1, Lineage 1 via Acquisition of the oxa23 Carbapenem Resistance Gene and Dispersion of ISAba1	abs# 243
Marwa M. Hassan	Detection and quantification of the heterogeneous S. aureus populations to identify antibiotic-induced persistence	abs# 244
Behnaz Heydarchi	Optimisation of antibody effector functions in HIV neutralising monoclonal antibodies	abs# 245
Jennifer Hosmer	Host-derived lactate is an important Haemophilus influenzae carbon source	abs# 246
Casey K Huang	The enemy of my enemy is my friend: eliminating Legionella in premise plumbing biofilms using bacteriophages	abs# 247
Seon Do Hwang	Recent Laboratory Findings of Brucella abortus in South Korea	abs# 248
Zahra Islam	Atmospheric trace gas oxidation supports persistence of the environmentally abundant phylum Chloroflexi	abs# 249
Muhammad Fahmi Ismail	Evaluation of an RNA extraction control for routine laboratory-developed reverse transcriptase-PCR diagnostic tests	abs# 250
Rhys Izuagbe	A prostate cell line model for persistent Zika virus replication	abs# 251
Megan Petterson	International Collection of Microorganisms from Plants (ICMP)	abs# 252

Poster Session 2

Agnes dela Cruz	Evaluation of porcine circovirus type 2 (PCV2) infection in pigs by histopathology, IHC and qPCR in tissue and serum samples from the Philippines	abs# 301
Francisco Garcia-Del Portillo	Peptidoglycan enzymes used by Salmonella inside eukaryotic cells	abs# 302
Arjuna N Ellepola	Changes in susceptibility of oral Candida dubliniensis isolates to lysozyme and lactoferrin following brief exposure to drugs with antifungal properties	abs# 303
Catherine Janto	One hour detection of organisms from positive blood culture broth using the BioFire FilmArray® Blood Culture Identification Panel	abs# 304
Catherine Janto	One hour detection of clinically significant pathogens from cerebrospinal fluid using the BioFire FilmArray® Meningitis/Encephalitis Panel	abs# 305
Yeongyun Jung	The prebiotic effects and human gut alteration of Gwakhyangjeonggisan, a traditional Korean medicine	abs# 306
Mwila Kabwe	Isolation and initial characterisation of bacteriophages against clinical isolates of Aeromonas hydrophila	abs# 307
Seong San Kang	Variability of top 7 Shiga toxin-producing Escherichia coli (STEC) and microbial populations through slaughter in Australian beef export abattoirs	abs# 308
Mohammad Katouli	Microbial diversity profiling and taxonomy evaluation of macropods using three hypervariable regions of bacterial 16S rRNA gene	abs# 309
Mohammad Katouli	Comparative genome analyses of Nocardia seriolae strains isolated from outbreaks of nocardiosis in fish farms in Vietnam	abs# 310
Johanna J Kenyon	Acinetobacter baumannii K24 capsular polysaccharide requires a novel gene for sugar biosynthesis and a gene located outside of the K locus	abs# 311
Karen HK Kong	Combating multidrug-resistant bacteria by phages equipped with sRNAs	abs# 312
Victoria Korolik	A chemoreceptor in Campylobacter jejuni responds to multiple classes of chemoeffectors	abs# 313
Tuan Son Le	Bacteriophages as a biocontrol agent of Vibrio species contaminating algae cultures in aquaculture	abs# 314

Poster Session 2

Inhyung Lee	Whole genome analysis of Aspergillus sojae SMF 134 supports its merits as a starter for soybean fermentation	abs# 315
Kyeong Ah Lee	Human brucellosis by Brucella melitensis in South Korea	abs# 316
Wai Leong	Mechanisms of intracellular survival of Pseudomonas aeruginosa in Acantamoeba castellanii and raw 264.7 macrophage cells	abs# 317
Jessica M Lewis	Unlocking the Complete Arsenal of Acinetobacter baumannii Type VI Secretion System Effectors	abs# 318
Yenkai YKL Lim	The performance of an oral microbiome biomarker panel in predicting oral cavity and oropharyngeal cancers	abs# 319
Yu-Wei Lin	Global Metabolomic Analysis of the Synergistic Killing against Extensive Drug-resistant Pseudomonas aeruginosa by the Combination of Polymyxin B and Enrofloxacin	abs# 320
Alison WS Luk	The carbohydrate dimension of nutrition in gut microbiome modulation	abs# 321
Dane Lyddiard	Searching for the remarkable among the unremarkable: Neglected plants from the New England region (NSW) and their antibacterial compounds	abs# 322
Stephanie Lynch	Characterisation of a Novel Bacteriophage and its Use in Therapies Against Bacteria Associated with Bovine Mastitis	abs# 323
Ian Macreadie	Statins promote clearance of Alzheimer's amyloid beta fused to green fluorescent protein in yeast	abs# 324
Jayne Manning	Non-capsular antibodies reduce pneumococcal colonisation density following therapeutic administration of pneumococcal whole cell vaccine	abs# 325
Arthika Manoharan	Disrupting and killing of Methicillin-resistant Staphylococcus aureus in biofilms	abs# 326
Andrea R McWhorter	A long-term efficacy trial of a live, attenuated Salmonella Typhimurium vaccine in layer hens	abs# 327
Andrea R. McWhorter	Exploring the behaviour of Salmonella Typhimurium in aioli, a raw egg-based sauce	abs# 328
Nway K. Mone	Molecular characterization of porcine circovirus type 2 in South East Queensland pig herds, Australia	abs# 329
Jean M Moselen	Evolutionary changes in the capsid P2 region of the norovirus GII.Pe_GII.4	abs# 330

Poster Session 2

Jean M Moselen	Organoids, a new, innovative way to grow "unculturable" human viruses that pose a public health threat	abs# 331
Talia S Moyle	The effect of in vivo passage on S. Typhimurium MLVA type and virulence	abs# 332
Tsitsi D Mubaiwa	Lectin activity of the Bexsero Neisseria meningitidis serogroup B vaccine antigen NHBA	abs# 333
Christopher A Mullally	Meningococcal Carriage Study of Young Adults in Western Australia	abs# 334
Joanna E Musik	The Sequence of the Mature Region of Secreted Proteins Impacts Signal Peptidase 1 Processing	abs# 335
Buddhie S. Nanayakkara	Ins and outs of blooming Escherichia coli	abs# 336
Marufa Nasreen	Extracytoplasmic Methionine sulfoxide reductase protects Haemophilus influenzae from oxidative stress caused by HOCl	abs# 337
Parisa Noorian	Genes involved in protozoan grazing resistance of Vibrio Vulnificus	abs# 338
Paraic O Cuiv	Forward genetics of the human pathobiont Bacteroides vulgatus reveals a novel mode of haem-iron acquisition	abs# 339
Malin M Olsson	Identification of novel antimicrobial compounds from Australian Myrtaceae species	abs# 340
Lida Omaleki	Squirrel gliders in the pasteurellosis spotlight	abs# 341
Cheryl-Lynn Y. Ong	The role of zinc acquisition and zinc tolerance in Group A streptococcal infection	abs# 342
Stephanie Owen	Identifying changes in painted and polyester surfaces during the attachment and proliferation of three species of environmental fungi using Synchrotron-sourced macro ATR-FTIR microspectroscopy	abs# 343
Chang Eon Park	Root microbial Dynamics influenced by Quorum-Sensing Signal Molecules on Continuous Cropping Soils of Ginseng	abs# 344
Amy Pham	The Prevalence of Inhibitory Antibodies in an Australian Cystic Fibrosis Cohort	abs# 345
Huong Pham	Regulation of c-di-AMP levels during osmotic stress in bacteria	abs# 346
Zachary N Phillips	The role of phase-variable epigenetic gene regulation in Streptococcus pneumoniae pathobiology and vaccine development	abs# 347

Poster Session 2

Alexander P Pintara	Molecular prediction of the O157:H-negative phenotype prevalent in Australian STEC patients	abs# 348
Katherine L. Pintor	Accumulation of Total Lipids and Triacylglycerides as Biodiesel Precursors upon Co-culturing a Phycospheric Bacterium with Indigenous Chlorella vulgaris	abs# 349
Miranda E Pitt	Evaluating the Extensively Drug-Resistant Klebsiella pneumoniae Resistome via MinION Sequencing	abs# 350
April Pottinger	Resistance for Dummies	abs# 351
Anggia Prasetyoputri	Investigation into resistance acquisition mechanisms in methicillin-resistant Staphylococcus aureus (MRSA) by means of resistance induction and next-generation sequencing	abs# 352
Arthur Prudêncio de Araujo Pereira	Mixed Eucalyptus and Acacia plantations: assessing the link between bacteria community and C-N functions in the soil and litter interface	abs# 353
Behrad Radmehr	Prevalence, Characteristics and behaviour of bacterial dairy contaminants	abs# 354
Munazza Rajput	Functional analysis of O-antigen modifying enzyme, O-acetyltransferase B, of Shigella flexneri	abs# 355
Josh T Ravensdale	Rapid Detection of Microbes Using DNA-PNA Hairpin Probes and the DiSC2(5) Dye	abs# 356
Alan J Rogers	Pseudomonas stutzeri skin and soft tissue infection with bacteraemia	abs# 357
Niloufar Roshan Hesari	Mechanisms of action of plant extracts against clostridium difficile	abs# 358
Niloufar Roshan Hesari	Effect of natural products on the production and activity of Clostridium difficile toxins in vitro	abs# 359
Tom Ross	Predicting VP meat quality and spoilage in domestic and international supply chain	abs# 360
Eileen Roulis	Investigating levels of parvovirus B19 DNA and genotypes circulating in Australian blood donors	abs# 361
Lina Rustanti	Potential to analyse bacterial contamination of platelets utilizing 16S metagenomics	abs# 362
Sumeet Sandhu	Bacterial communities vary between healing and non-healing diabetic foot ulcers (DFUs) - An update on the DFU microbiome	abs# 363

Poster Session 2

Amreeta Sarjit	Salmonella response to heat and pH in meat juice	abs# 364
Derek S Sarovich	ARDaP: Improved antimicrobial resistance detection and prediction from whole-genome sequence data	abs# 365
Shabana Shabana	Silver Nanoparticles: Green Synthesis and their Effective Applications	abs# 366
Susan Sharp	Comparison of Copan WASPTM versus BD KiestraTM InoqulA in Examination Time for Positive Urine Culture	abs# 367
Lucy K Shewell	Engineering a bacterial toxin for improved function as a N-glycolylneuraminic acid specific lectin	abs# 368
Samuel Dominggus Chandra Siahaan	Uropathogen Detection Comparison of Enhanced Quantitative Urine Culture Protocol and Standard Urine Culture Protocol in Patients with Urinary Tract Infection: Evidence Based Case Report	abs# 369
Reema Singh	Potential pathogens identified in lung samples from pigs with pleurisy at an abattoir in Queensland Australia	abs# 370
Gavin JD Smith	Investigating the risk of human disease from parasites of small mammals and bats in Cambodia	abs# 371
Liesel Stassen	High susceptibility and vector competence of Australian Aedes aegypti to Zika virus	abs# 372
Lisa Stinson	Seeding of the fetal gut microbiome: insights into origins and significance	abs# 373
Miljan Stupar	Molecular characterisation of PPVP, a key effector molecule within the LirA regulon of Mycobacterium tuberculosis	abs# 374
Yvonne Su	Evolution of influenza A/H3N2 virus in Singapore	abs# 375
Aroon Supramaniam	Heparan sulfate mimetic compounds in modulating RRV as potential therapies	abs# 376
Christopher D Swan	Faecal Strongyloides stercoralis real-time polymerase chain reaction assay validation study	abs# 377
Aimee Tan	Nuc is an extracellular nuclease that mediates competence and biofilm formation in Moraxella catarrhalis	abs# 378
Fallen Teoh	Adaptation Strategies Inferred From Resource Allocation in Cyanobacterial Membrane Proteomes	abs# 379
Priya Thaivalappil Thotsapol	Comparison of the highly multiplexed PlexPCR RespiVirus 11 (beta) assay with a commercial assay Molecular characterization of fluoroquinolone-	abs# 380

Poster Session 2

Thomrongsuwannakij	resistant avian pathogenic Escherichia coli isolated from diseased chickens in Thailand	abs# 381
Sarah Tozer	Overdiagnosis of rotavirus infection due to vaccine virus shedding	abs# 382
Sarah J Tozer	Are peri-urban wild dogs another potential source of transmission of Q fever in Queensland?	abs# 383
Merrin Tulloch	Study for Monitoring Antimicrobial Resistance Trends (SMART) in Australia and New Zealand (ANZ), 2016-2017	abs# 384
Conny Turni	Glaesserella australis sp. nov., isolated from the lungs of pigs	abs# 385
Md Jalal Uddin	Phenotypic and genotypic variability of antibiotic-resistant Salmonella Typhimurium exposed to environmental stresses	abs# 386
Md Jalal Uddin	Characterization of antibiotic-resistant Staphylococcus aureus exposed to oxacillin and ciprofloxacin	abs# 387
Md Jalal Uddin	Effect of bacteriophage-antibiotic combination on the reduction of the development of antibiotic resistance in Salmonella Typhimurium	abs# 388
Jay Prakash Verma	Development of indigenous novel climate resilient microbial consortium for enhancing vegetable production	abs# 389
Thu NM Vu	Funcional studies of pyruvate carboxylase regulation by cyclic di-amp in lactic acid bacteria	abs# 390
David L Wakeham	Use of whole genome sequencing to determine the genetic basis of multidrug resistance in Escherichia coli isolated from Australian livestock	abs# 391
Liang Wang	Could environmental durability act as an enabler of bacterial pathogenicity?	abs# 392
Nimsha S Weerakkody	Evaluation of cytotoxicity levels and synergistic antimicrobial activity of Alpiniamalaccensisand Terminaliacatappa extracts against food borne or spoilage bacteria in vacuum packed ready-to-cook (RTC) chicken	abs# 393
Rhys T White	Capsule-switching is associated with the rapid global expansion of the recently emerged fluoroquinolone-resistant Escherichia coli sequence type 1193 clone	abs# 394
Gal Winter	Soil and the gut microbiota- Supporting the “hygiene hypothesis”	abs# 395
Ka Yan Wong	Evaluation of the Vitek 2 AST YS08 Yeast Susceptibility Test in comparison to the Sensititre YeastONE Susceptibility System for antifungal susceptibility testing	abs# 396

Poster Session 2

Alma Wu	Functional Diversity of Toxin-Antitoxin Systems in Antibiotic Resistance Plasmids in Enterobacteriaceae	abs# 397
Sarah Yee	The molecular epidemiology of an atypical wooden tongue outbreak	abs# 398
Sung-il Yoon	Structural study of a molybdenum cofactor-dependent enzyme	abs# 399
Lauren Zavan	Growth stage of Helicobacter pylori regulates the production and content of outer membrane vesicles	abs# 400
Xiaomei Zhang	Identification of serovars’ specific genes for typing the five most prevalent Salmonella serovars in Australia	abs# 401
Jinxin Zhao	Metabolomic analysis uncovered the synergistic mechanisms of polymyxin B in combination with rifampicin against MDR Acinetobacter baumannii	abs# 402
Marina L Zupan	Elucidating the Zn(II)-binding mechanism of the pneumococcal protein AdcAII	abs# 403

Author Index

Ambu, S	206
Andrianova, E.P	313
Counoupas, C	42
Ginsberg, R	89
Papudeshi, B	229
Rankine-Wilson, L	205
Thomson, N	98
Abell, S	164
Abeysekera, D.M	201
Abraham, S	391
Abramov, T	314
Acharya, D	202
Adams, J	159
Adams, V	147
Ahmed, A	366
Ahmed, W	309
Ahn, J	231,386,387,388
Akbarsha, A	393
Alawneh, J.A	329
Aleer, S	6
Allerton, J.K	203,204
Alsuwayyid, B	205
Amalraj, F	206,227
Anandan, A	45
Anderson, D	106
Anderson, J	374
Andrianopoulos, A	143
Ansell, B	20
Aquino, N.L	207
Asaye, M.A	161
Asgari, S	85,86
Ashcroft, M.M	208,391,394
Astley, D	8
Atack, J	79
Atack, J.M	347
Atkins, T	32
Augustin, M	238
Awad, M.M	10,147
Aye, S	209
Aziz, A	210
Badal, R	384
Bainomugisa, A	352
Baker, B.J	15
Baker, L	20
Baker, M	115
Baker, R.E	135
Balch, R	66
Balgahom, R	304,305
Bansal, N	346,390
Barker, D	47
Barker, S.C	47
Barlow, R	308
Barnes, T	301,370
Barnes, T.S	329
Barr, J	144,145
Barratt, K	380
Bartlett, Z	31
Bartley, P	101
Barugahare , A	14
Beale, D	238
Beatson, S	144
Beatson, S.A	105,12,149,208,219,391,394
Beatson, S.A	341,78
Beddoe, T	323
Begg, S.L	211
Begg, S.L	34
Begun, J	339,7
Behruznia, M	212
Beilharz, T	140
Beissbarth, J	210
Bell, J	213,394,6
Bell, J.M	214
Bell, S	127,345
Bell, S.C	104
Bell, S.M	203,204
Ben Zakour, N	98
Berendsen, E.M	215
Bew, J.D	72
Bhandari, M	216
Bhattacharya, D	99
Bialasiewicz, S	159,236,66
Binti Mohammad Abdul Aziz , A	227
Birikmen, M	365
Bitto, N	73
Bitto, N.J	217,400
Blackall, P	111,112,218,370,385,398,60,79
Blackall, P.J	93
Blackall, P.J	329,341,78
Blakeway, L.V	378
Blaskovich, M.A	350,352
Bletchly, C	382
Bloomfield, L	334
Blyth, C.C	106
Boan, P	396
Bojesen, M	218
Bond, P	144,247
Bongcaron, V.R	325
Borthwick, S	371
Botella, J	111
Bowman, J	241
Bowman, J.P	110
Bowring, B	98
Boyce, J	242
Boyce, J.D	14,221,318
Boyd, B	100
Brandon, R	65
Branley, J	160,304,305
Bransgrove, K	164
Branston, S	98
Bray, J.E	72
Brazel, E.B	34
Bretag, T	91
Britton, W	42
Brockway, A	30
Brooks, A	114
Brooks, P.R	340
Brown, M	159,16
Brown, N.F	152
Brown, T	307
Bruggink, L.D	330
Brunetti, G	6
Buckley, C	219
Buckley, S.J	220
Buller, N.B	341
Buller, N	391
Bullivant, M	207
Bumbak, P	106
Burbridge, P	325
Burch, M.A	221
Burton, D	1
Bustamante, A	222
Butler, M.S	244
Bzdyl, N	97
Bzdyl, N.M	32
C. Southgate , P	314
Cabezas, S	113
Caly, L	331
Cao, M	352
Carere, C	249
Carman, R	68
Carney, R	16
Carr, J	113
Carrick, J	77
Carter, D.A	30
Carver, S	160,44
Castanheira, S	302
Catherine,	206
Cervin, A	159
Cestero, J	302
Chamoun, M.N	223
Chan, H	307
Chan, J.F	117
Chan, K	117
Chandar, B	99
Chandry, S	238,241
Chandry, S.P	404
Chang, A	210
Chang, B.J	125,239
Chansiripornchai, N	381
Chen, S	124
Chen, S.H	110
Cheng, L	217
Cheng, V.C	117
Chheang, D	371
Chilton, A.M	39
Chiow, A	141
Choi, Y	315
Chong, S	234
Choong, N	234
Chousalkar, K.K	327,328,332
Chow, W	117
Christensen, H	385
Christophi, C	331
Chu,	206
Chu , W	227
Clark, J	382
Clements, A	4
Coates, H	106
Cobbold, R	383
Coenye, T	131
Coggon, C.F	33
Coin, L	352,66
Coin, L.J	350
Coleman, A	159
Collins, D.A	224
Connolly, J.H	163
Conroy, T	34
consortium, T	232
Constancias, F	18
Convey, P	206,227
Coombs, G	205,384,97
Coombs, G.W	72
Cooper, M.A	350,352
Cooper, M.A	244
Cooray, R	404
Coorey, R	356,364
Cormican, M	201
Cormie, C	148
Cottrell, K	159
Coulibaly, F	148
Crawford, R	167
Crayn, D	164
Creek, D	320,402
Creek, D.J	209
Crosbie, N	20
Cuddihy, T.P	78
Cunningham, B.A	10
Cuomo, C	30
Currie, B.J	104
Daly, J	132
Daniel , C	206
Darbo, J	86
Das, A	326
Davies, C.B	225
Davies, M	220
Davis, J	94
Day, C	153
Day, C.J	313,333,335,368
De Souza, D.P	161
Dekker Nitert, M	136
dela Cruz, A	301
Deng, L	132
Denman, S	36
DeSoyza, A	127
Deveson Lucas, D	221,242,318
Devine, G	86
Devine, G.J	372
Devlin, J	75
Dewi, D	226
Dharshini, D	227
Dhouib, R	129,216,228,337
Diane Maresco-Pennisi, D	159
Dilcher, M	380
Dinsdale, E.A	229
Djordjevic, J	139
Doane, M.P	229

Author Index

Author Index

Dombrowski, N	15
Donner, E	6
Dougan, G	148
Drigo, B	6
Drinkovic, D	384
Drinkwater, N	10
Du, Y	392
Duarte, T	66
Duarte, T.P	350
Duchêne, S	123
Duell, B	202
Duffy, L	108
Duffy, L.L	110
Dunne, E.M	325
Dunstan, R	148
Durrer, A	353
Dutilh, B.E	232
Dykes, G	308
Dykes, G.A	230,356,364,404
Dzianach, P.A	230
Dzunkova, M	132
Easwaran, M	231
Edwards, J	245,56
Edwards, R.A	232
Effler, P	334
Eijkelkamp, B.A	211
Eijkelkamp, B.A	34
Ekwudu, O	372
El-Deeb, I.M	34
Elango, D	151
Elgamoudi, B.A	313
Ellem , J	384
Ellepola, A.N	303
Elsner, A	207
Eme, L	15
Emes, R	37
Eng, L	233,234
Epstein, V	398
Erken, M	18
Espinosa-Gongora, C	218
Espinoza Vergara, G	50
Ettema, T.J	15
Evans, G	45
Fabijan, J	37
Faddy, H	361,362
Faddy, H.M	236,372
Fallowfield, H	109
Fantino, E	129
Faryal, R	324
Fegan, N	110,364
Feng, J	249
Ferguson, J	159
Fernandes, K.E	30
Fernandez Caceres, E	15
Ferro, V	376
Fifis, T	331
Filipovic, I	86
Firdaus, R	235
Fitzsimons, T.C	318
Flanagan, D.J	331
Flower, R	361,362
Flower, R.L	236
Fong, W	124,237
Forbes, K.J	230
Forde, B.M	105,12,149,208,219,391,394
Forde, B.M	341,78
Fowloer, E.E	58
Fox, D	97
Fox, E	241
Fox, E.M	238
Frentiu, F	251
Frentiu, F.D	236,372
Froumine, R	123
Fukuma, N.N	319
Fuller, K	32,45
Furfaro, L.L	125,239
Galani, I	209
Ganesamoorthy, D	352
Gantier, M.P	81
Garcia-Del Portillo, F	302
Gardam, D	396
Gardiner, K	324
Gaurav, A	389
George , N	384
Ghorashi, S	163
Giamarellou, E	209
Giang, N	385
Gibson, J.S	391,394
Gilbert, R	38
Gilbert, R.A	35
Giogha, C	152
Giri, R	7
Givney, R	64
Glasbey, T	326
Gleason, F.H	165
Goh, K.G	33
Goh, K.G	12
Goldberg, D.E	21
Goldblatt, D	325
Gonelli, C	245,56
Gordon, D	212
Gordon, D.L	113
Gordon, D.M	336
Gorman, E	361
Gorman, E.C	236
Gorrie, C	123
Goulding, D	148
Govan, B	158
Grahn Håkansson, E	159
Graves, S.R	48
Graves, S.R	240
Gray, J	241
Greatrex, B.W	322
Greening, C	249
Greening, D	400
Gregory, M.K	211
Grimwood, K	128,382
Grinter, R	148

Author Index

Guglielmino, C.J	348
Gulliver, E	14
Gulliver, E.L	242
Gumiere, T	353
Haggerty, J.M	229
Hair, S.D	341
Hajkowicz, K	66
Hall, R.M	243,311,70
Hamidian, M	243
Hamilton, A.L	215
Hammer, K	358
Hammer, K.K	359
Han, M	209,320,402
Hancock, S.J	149
Hardy, J	148
Haremza, E	207
Harmer, C.J	70
Harper, M	14,221,242,318
Harper, R	91
Harrich, D	116,57
Harriott, L	383
Harris, P	120
Harris, T.M	210
Harrison, M.D	225
Harrison, P	140
Hartland, E.L	152
Hartley, C	144
Hartley-Tassell, L	333
Hartley-Tassell, L.E	313
Harvey, R	154
Haselhorst, T	153
Hasenkopf, A	97
Hassan, M.M	244
Haverkamp, M	30
Hawkey, J	243
Hay, I.D	25
Helbig, K	323
Heller, J	40
Hemmatzadeh, F	37
Henderson, A	105
Henderson, I.R	127,33
Henderson, L	165
Heney, C	66
Henningham, A	129
Heras, B	11,228,29
Herisse, M	96
Herrero, L	376,51
Herrero, L.J	118
Hewitson, J	112
Heydarchi, B	245,56
Hick, P	19
Hickey, M	140
Hill, A	217
Hill, A.F	400
Hill, R	80
Hitch, A	371
Hoad, V.C	236
Hoang, M	142
Hoedt, E.C	215
Hogan, T	219
Holmes, A	321
Holmes, A.J	67
Holt, K.E	123,243
Holzgrabe, U	97
Hong, S	68
Hosmer, J	129,246
Hovel, K	229
Howard, P	124
Howden, B.P	96
Howes, M.T	166
Huang, C.K	247
Hubber , A	384
Hugenholtz, P	132
Hugo, L.E	372
Hurh, B	315
Hurst, M.R	52
Hutton, M	68
Hutton, M.L	10,62
Huygens, F	225,348,363,65
Hwang, S	248,316
Ibrahim, Y	233
Imbrogno, K	106
Inglis, T.J	32
Ipe, D.S	223
Iqbal, H	26
Iredell, J	397,74,98
Irinyi, L	142
Irwin, P	49
Islam, M	160
Islam, Z	249
ISMAIL, M	250
Ivanova, E.P	167
Iwasaki, J	97
Izuagbe, R	251
Jackson, B	398
Jaiswal , D	389
James, P.M	203,204
Jamieson, S.E	106
Janto, C	304,305
Jarvis, G.A	45
Jelocnik, M	160,77
Jen, F.E	153
Jen, F.E	155
Jenkins, C	160,77
Jenney, A	123
Jennings, M	137,153,28,368,79
Jennings, M.M	155
Jennings, M.P	333,335,347
Jennison, A	112,69
Jennison, A.V	121,348
Jenull, S	138
Jeon, G	386,387
Jeppesen, M	240
Jex, A	20
Jiang, A	33
Jin, H	57
John, C	45
John, N	20

Author Index

Johnson, K.N	85
Johnston, E	73
Jolley, K.A	72
Jones, M	40
Jones, S	304
Jordan, D	391
Jorgensen, S.L	15
Judd, L.M	123
JUNG, Y	306,344
Jurandy Bran Nogueira Cardoso	353
Kabwe, M	307
Kacev, D	229
Kadalamani, B	393
Kahler, C	205,32,97
Kahler, C.M	125,334,392,45,72
Kaler, J	37
Kama, M	5
Kamm, M.A	215
Kamruzzaman, M	397,74
Kang, B	248,316
Kang, S	308
Kappler, U	129,246,337
Karaiskos, I	209
Katouli, M	309,310,8
Kaur, M	241,360
Kavanagh, A	352
Keelan, J	373
Keerthiratne, T.P	109
Keil, A	130
Keil, A.D	72
Kelly, W.J	35
Kenny, L.L	319
Kenyon, J.J	311
Kerr, E.D	151,166
Ketheesan, N	158
Khan, Z.U	303
Khoo, C	161,97
Khoury, G	56
Kibble, E	97
Kidd, T	71
Kidd, T.J	104
Kidsley, A	394
Kim, J	248,316
Kim, K	315,315
Kim, M	248,316
King, R.M	313
Klieve, A	38
Klieve, A.V	35
Knibb, W	310
Knight, D	358
Knight, D.D	359
Knight, D.R	3,68
Kobe, B	149,403
Kocharunchitt, C	110,241
Kok, J	375
Kong, K.H	312
Korman, T	384
Korolik, V	153,313
Kovach, Z	98
Krause, L	86
Kuballa, A	309,8
Kuchler, K	138,168
Kumar Singh, B	353
Kurtboke, D	310,314
Kyaw-Tanner, M	329
Labbate, M	16
Lahra, M	69
Lahra, M.M	219
Lam, P	375
Lambert, S	382
Lammers, G	40
Lan, R	130,40,401
Lappan, R	106
Lau, S.K	117
Lau, T.C	312
Le, C	310
Le, T	314
Ledbetter, J	229
Lee, H	375
Lee, I	315
Lee, K	248,316
Leong, N	233
Leong, W	317
Lev, S	139
Lewis, J.M	318
Li, D	116,57
Li, J	209,320,402
li, l	57
Li, W	378
Liaskos, M	217,400,41,73
Licciardi, P	325
Licona-Cassani, C	46
Lilje, O	165
Lim, E.X	118
Lim, Y.Y	319
Lim, Y	306
Lima, N	380
Lin, Y	320,402
Lithgow, T	148,25
Liu, Q	392
Liu, Z	392
Lizarraga, D	44
Lo, A	11,13,149
Lo, R	346,390
Lo, T	140
Loessner, D	251
Lombard, J	15
Lombi, E	6
Londrigan, S	114
LOO, L	250
Looke, D	105
Lor, M	57
Lorimer, D	97
Low, S	132
Lu, T	144
Luk, A.W	321
Luo, Z	149,403
Lutz, C	317

Author Index

Luu, L	130
Lyddiard, D	322
Lynch, S	323
Lyras, D	10,147,62,68
M Rajan, S	380
Macdonald, J	53
Machalek, D.A	55
Macia, L	321
Mackenzie, C	56
MacLaughlin, S	167
Macreadie, I	324
Madden, D	160,365
Mahony, T	80
Mahony, T.T	58
MAIWALD, M	250
Mak, J	153,54
Malley, R	325
Manna, S	59
Manning, J	325
Manoharan, A	326
Manos, J	326
Marano, A.V	165
Marcellin, E	346,46
Marsh, R	126
Marshall, J.A	330
Martin, J	228
Martinez, E	222
Mason, M	111
Masters, N	309,8
McAllister, L	154
McCosker, K	38
McDevitt, C.A	211,403
McDevitt, C.A	34
McDonald, M	162
McDougald, D	18,317,338,50
McEwan, A	129,246
McEwan, A.G	337,342
McEwan, A.G	34
McGowan, S	10
McGrath, E	201
McKenzie, P	370
McKew, G.L	377
Mcmillan, D.J	220,340
McWhorter, A.R	327,332
McWhorter, A.R	328
Meers, J	301,329,37,370
Meetam, M	349
Mégroz, M.N	14
Meleady, K	213
Melki, J.R	201
Mendenhall, I	375
Mendenhall, I.H	371
Meyer, W	142
Millar, D.S	201
Minhas, V	154
Mohamad, N	206
Mok, D	106
Mokany, E	380
Mollinger, J.L	391,394
Monahan, B	143
Mone, K	370
Mone, N.K	329
Monsanto-Hearne, V.M	85
Moonen, G	46
Moore, S.C	238
Moraes Gonçalves, J	353
Morales, S	98
Morey, F	92
Morona, R	153
Morris, M	229
Morrison, M	136,215,339,7
Morrison, M.M	319
Moselen, J.M	330,331
Moseley, G	23
Moutafis, G	46
Mowlaboccus, S	334,72
Moyle, T.S	328,332
Mubaiwa, T.D	153,333
Mulhern, T	89
Mulholland, K	325
Mullally, C.A	334,45
Muller, B	56
Muñoz-Rojas, M	39
Murthy, A	13
Musik, J.E	335
Myler, P	97
Naderer, T	140
Nainggolan, G	369
Nanayakkara, B.S	336
Nasreen, M	129,246,337
Negara, W	38
Neha, N	161
Neilan, B.A	39
Nelson, J	367
Newham, S	20
Newson, J.P	152
Newton, H	97
Newton, H.J	161
Ng, L	233,234
Ng, S.C	215
Nguyen, C	240
Nguyen, N	13
Nguyen, S	350,66
Nhiep, N	346
Niamsuphap, S	151
Nimmo, G	382,69
Noorbakhsh, M.H	367
Noorian, P	338,50
Norton, R	157,158
Norville, I	32
Nosal, A	229
Nosworthy, E	210
Novak-Weekley, S	367
O Cuiv, P	136,339,7
O'Callaghan, M	52
O'Dea, C	309
Octavia, S	130,40
Offre, P	134

Author Index

Ogbourne, S.M	340
Oldfield, N	235
Olsson, M.M	340
Omaleki, L	341,385,78
Ong, C	342
Orellana, C	46
Ostrowski, M	379
Ouwerkerk, D	35,38
Owen, H	37
Owen, S	167
O'Connor, W	314
Palmieri, C	301
Palombo, E	354
Pande, D	229
Park, C	344
Parke, C	301
Parke, C.R	329
Parke, K	370
Parra-Saldivar, R	26
Parry, R	86
Pasic, L	142
Paterson, D.L	105,149
Pathirana, E	19
Pathirana, R	217
Paton, A.W	368
Paton, J	154
Paton, J.C	211,347,368,403
Paton, J.C	34
Paulsen, I	379
Paulson, A.R	52
Paxman, J	228,29
Paxman, J.J	11
Payne, M	130,373,401
Payne, M.S	125,239
Peacock, C.S	106
Pearson, J.S	152
Pearson, J	205
Peat, T	144
Pederick, V.G	211,403
Pegg, C.L	151
Peleg, A	61
Peleg, A.Y	221
Pell, C.L	325
Pepin, G	81
Pereira , A	389
Pérez-Reche, F.J	230
Perfect, J	30
Perkins, N	383
Peters, K.M	149,208
Petrovski, S	307,323,73
Petterson, M	252
Pham, A	127,345
Pham, H	346
Pham, H.T	390
Pham Quang, H	344
Phan, M	12,149,208
Phan, T	377
Phillips, Z.N	347
Pickard, D	148
Pidot, S.J	96
Pintara, A.P	348
Pintor, K.L	349
Pitt, M.E	350,352
Playford, G	105
Pluschke, G	155
Polkinghorne, A	160,77
Pong, C	70
Poo, Y.S	86
Pottenger, S	136,7
pottinger, a	351
Powell, D	14,221,242,310
Power, J	46
Prasetyoputri, A	352
Premakumara,	393
Prestidge, C.A	100
Price, E	310
Price, E.P	365
Price, E.P	104,210
Price, G	158
Prudêncio de Araujo Pereira, A	353
Pucciarelli, M	302
Pumtang-on, P	80
Punyadeera, C.C	319
Purcell, D	245
Purcell, D.F	56
Quan, D	42
Radmehr, B	354
Rafferty, D.L	203,204
Rahman, H	313
Rainey, P.B	52
Rajput, M	355
Ramirez, D	229
Ramu, S	352
Ranaweera , K	393
Ranzoni, A	244
Rasic, G	86
Rathnayake, I	363
Rathnayake, I.U	348
Raubenheimer, D	321
Ravensdale, J.T	356,364,404
Rawle, D.J	116
Read, M.N	67
Reading, P	114
Reed, H	339
Reid, S	5
Rice, D	323
Richter, K	100,131
Riedelberger, M	138
Riley, T	358
Riley, T.T	359
Riley, T.V	224,68
Rinke, C	132
Roberts, J.A	330
Roberts, L.W	391,394
Roberts , S	384
Robertson, G	156
Robins-Browne, R.M	325,340
Robson, J	240

Author Index

Roddam, L	103
Rodrigues, C	146
Rogers, A.J	357
Rogers, B.A	394
Rood, J.I	147
Roos, K	159
Rosch, J.W	34
Roshan Hesari, N	358
Roshan Hesari, N.N	359
Ross, K	109
Ross, P	240
Ross, T	360
Roulis, E	361,362
Rudd, P	51
Rudd, P.A	118
Rudd, S	363
Rush, C	158
Rustanti, L	362
Ruter, R	62
Rutledge, P	42
Sadsad, R	124
Salazar-Quiroz, N	56
Samarasekara, H	304,305
Sandhu, S	363
Sansom, F.M	161
Sarjit, A	364
Sarkar, S	62
Sarkar-Tyson, M	32,97
Sarker, N	37
Sarovich, D	310
Sarovich, D.S	365
Sarovich, D.S	104,210
Sassetti, C.M	135
Satzke, C	325
Scanlon, M	228,45
Schaale, K	13
Schabacker, K	365
Schatz, T	38
Schembri, M	11,13,144,202
Schembri, M.A	12,149,208,223,33,391,394,62
Schirra, H	129,246
Schittenhelm, R.B	221,242
Schüller, S	62
Schulz, B.L	155
Schulz, B.L	151,166
Schwab, R.H	331
Scott, A	32
Scott, N	152
Seddon, J	37
Seemann, T	96
Seib, K	153
Seib, K.L	333,378,43
Selvarajah, S	206
Selvarajoo, K	141
Semchenko, E	153,43
Semchenko, E.A	333
Seymour, J	16
SHABANA, S	366
Sharma, P	228
Sharp, S	27,367,63
Sheehan, E	127
Shelley, D	314
Shewell, L.K	368
Shin, J	306,344
Short, M	6
Siahaan, S	369
Siboni, N	16
Sikazwe, C	106
Simmons, G	37
Singh, R	370
Singh, T	238
Singleton, J	10
Sintchenko, V	124,130,222,237,401
Sly, P	129
Smith, C.M	135
Smith, D	127,345
Smith, G	375
Smith, G.J	371
Smith-Vaughan, H	210
Snelgrove, S	140
So, J	306
Soares, D.C	116
Soares, R	383
Somarathna , T	393
Spang, A	15,88
Speers, D	205
Speers, D.J	72
Speight, N	37
Spillman, N.J	21
Sridhar, S	117
Srikhanta, Y.N	10
Stairs, C	15
Stassen, L	251,372
Steed, B	143
Stenos, J	240
Stephenson, E	51
Stewart, K	214
Stinear, T.P	217,96
Stinson, L	373
Stodart, B.J	163
Strachan, N.J	230
Stratton, H	309
Strugnell, R.A	149
Stubbs, K	45
Stupar, M	374
Su, Y	375
Subedi, P	29
Subhan, M	324
Suhrbier, A	86
Sukumaran, V	66
Sullivan, M	13,202
Sullivan, M.J	223,24
Sun, S	18,338,50
Supramaniam, A	376
Swan, C.D	377
Swedberg, J	116
Sweet, M	13
Sweet, M.J	12

Author Index

Sy, B	14,242
Tachedjian, G	82
Tan, A	378
Tan, J	317
Tan, L	380
Tan, T	233,234
Tarlinton, R	37
Tay, A	130
Taylor, A	84
Taylor , S	384
Taylor-Brown, A	309
Templeton, J	111,112,370
Teoh, F	379
Thaivalappil, P	380
Tham, A.L	85
Thomas, N	100,131
Thomas, T	226
Thompson, P	139
Thomrongsuwannakij, T	341,381
Thomson, R	102
Thorn, C	100
Timms, P	220,44
Timms, V	130,237
Timms, V.J	124
To, K.K	117
Tobin, M.J	167
Torres, V	87,9
Totsika, M	216,228,62
Totsika, M.M	319
Tozer, S	236,382
Tozer, S.J	383
Tran, B.M	331
Tran, E	153
Trapetti, C	211
Trappetti, C	154,347
Traven, A	140
Tree, J	242
Tree, J.J	14
Trembizki, E	69
Triccas, J	42
Trott, D	37
Trott, D.J	391,394
Trueman, S.J	340
Truong, V	167
Truszewskia, E	165
Tscherner, M	138
Tucci, J	307
Tucey, T	140
Tull, D	161
Tulloch, M	384
Turner, M	346,390
Turner, M.S	107
Turni, C	301,329,341,370,385,398,76,78
Turnidge, J	213,394
Turnidge, J.D	214
Tweedie, A	19
Uddin, M	386,387,388
Ukuwela, A	29
Ulett, G	13,202
Ulett, G.C	12,223,24
Vagenas, D	216,228,62
van den Hurk, A	83
van Ogtrop, F	30
Vanniasinkam, T	80
Velkov, T	209,320,402
Venter, R	6
Venturini, C	98
Verbruggen, H	133
Verma, J	353,389
Verma, N.N	355
Verma Gaur, J	140
Viberg, L.T	104
Vidor, C.J	147
Viennet , E	372
Vijayasekaran, S	106
Villalba-Rodríguez, A	26
Villalón-Letelier, F	114
Vincan, E	331
Virk, R	375
Vital, P.G	349
Vitali, S.D	341
von Itzstein, M	34
Vongsvivut, J	167
Vreugde, S	131
Vrielink, A	45
Vu, T	346
Vu, T.N	390
Vu-Khac, H	310
Wai Yew, C	227
Wakeham, D.L	391
Walker, D	207
Walker, M	95
Walker, M.J	342
Walker, M.J	34
Wallace, A.M	341
Walsh, T.R	149
Wang, J	353,368
Wang, L	116,392,57
Wang, Q	401
Wang, T	11,228,29
Ware, R	159
Watts, T.D	147
Wawegama, N.K	161
Webster, N	17
Weerakkody, N.S	393
Weir, B	252
Wells, T	345
Wells, T.J	127,33
West, N	374
Weynberg, K	144,247
Wheatley, V	90
Whiley, D	382,69
Whiley, D.M	219
Whiley, H	109
Whitchurch, C	22
White, R.T	394
Whiteley, G	326
Whittington, R	19

Author Index

Wick, R	243
Wick, R.R	123
Wickramasinghe, N	404
Wijburg, O.L	325
Williamson, D.A	122
Wimpenny, T.T	58
Winter, G	395
Wise, M	205
Wise, M.J	392
Wong, J	14,242
Wong, K	396
Wong, Z	85
Wong Fok Lung, T	152
Woo, P.C	117
Wood, A	159
Wood, C	383
Wooldridge, K	235
Woolford, L	37
Wu, A	397
Wu, V	385
Wu, X	6
Wyres, K.L	123
Xiao, Z	29
Xu, A	130
Xu, Z	57
Yan, J	392
Yang, J	149,340
Ye, S	382
Yee, S	370,398
Yildiz, F	150,2
Yip, C.C	117
Yoon, S	399
Young, P	119
Yu, J	215
Yuen, K	117
Zacchi, L.F	151
Zaferanloo, B	354
Zakrzewski, M	86
Zalucki, Y	137
Zalucki, Y.M	335
Zaragoza, N	46
Zavan, L	400
Zhang, F	215
Zhang, J	215
Zhang, X	401,52
Zhao, J	320,402
Zhao, P.S	117
Zhao-Xun, L	346
Zhu, Y	209,320,402
Zhulin, I.B	313
Zupan, M.L	403
Zwolanek, F	138

Oral Abstracts

1

Progress toward a neutralizing antibody-based HIV vaccine

Dennis Burton[2, 1]

1. Ragon Institute of MGH, MIT and Harvard, Boston, Massachusetts, United States of America

2. Dept of Immunology and Microbiology, The Scripps Research Institute, La Jolla, CA, United States

Highly antigenically variable viruses such as HIV present major problems for vaccine design. Broadly neutralizing antibodies to HIV generated during natural infection can identify weaknesses in the surface structures of the virus. These weaknesses can help guide vaccine and drug design and reveal fascinating aspects of the interplay between two highly mutable systems-the virus and antibody

2

Mechanisms and regulation of biofilm formation

Fitnat Yildiz[1]

1. University of California, Santa Cruz, Santa Cruz, CA, United States

Biofilms, surface attached microbial communities composed of microorganisms and the extra-polymeric substances they produce, enhance growth and survival of microorganisms in natural settings. Biofilms contribute to dissemination, environmental survival, and transmission of human pathogens. All aspects of biofilm formation, i.e. cell-surface interactions, biofilm matrix production, biofilm maturation and dispersal, are exquisitely controlled by diverse regulatory circuitries. The nucleotide based intracellular signaling molecule cyclic di-guanosinemonophosphate (c-di-GMP) is a central component of biofilm regulatory circuitries in bacteria. In this presentation, I will describe mechanisms of surface attachment and biofilm formation in *Vibrio cholerae*, the causative agent of the disease cholera. Additionally, I will describe how c-di-GMP governs motile-to-biofilm life transition and biofilm matrix production.

3

Clostridium difficile infection in Australia: not so nosocomial anymore

Daniel R Knight[1]

1. School of Veterinary and Life Sciences, Murdoch University, Perth, WA, Australia

Clostridium difficile infection (CDI) presents a major clinical and economic challenge to public health systems, reaching an epidemic state in some regions of the world. In Australia, increasing rates of community-acquired CDI (now accounting for 30% of all cases) suggest exposure to *C. difficile* reservoirs outside the hospital, including animals, food or the environment. Indeed, Australian livestock are a significant reservoir for evolutionary lineages of *C. difficile* causing CDI in humans. The amplification of *C. difficile* in production animal systems and subsequent contamination of meat, vegetables grown in soil containing animal faeces, and agricultural by-products such as compost and manure, is driving the insidious rise of CDI in the community.

One Health is a philosophical approach to improving and safeguarding the health of humans, animals and the environment and, importantly, recognises that these three areas are inter-related. In this regard, CDI is the quintessential One Health issue. The application of high-resolution microbial genomics in a One Health framework has yielded critical insights into the evolution and transmission of *C. difficile* in humans and animals, and their shared environment. This talk will provide an overview of our recent genomic investigations that provide substantive evidence of transmission between livestock and humans of two lineages of *C. difficile* of One Health importance (ribotype 014 and sequence type 11). These findings provide compelling evidence of a zoonosis and challenge the existing paradigm that CDI is exclusively a healthcare-associated infection.

4

The epidemiology of *Clostridium difficile* infections

Archie Clements[1]

1. Curtin University, Bentley, WA, Australia

We have addressed knowledge gaps in the epidemiology of *Clostridium difficile* using a programme of epidemiological research involving prospective molecular epidemiological surveys, mathematical modelling, economic studies, systematic reviews and meta-analyses. We demonstrated a very similar profile of PCR ribotypes of *C. difficile* in hospitals and the community. Modelling studies demonstrated that transmission of infections in the hospital is sustained by introduction of infections from the community, without which, infection in hospitals would die out. The current two-day cut-off defining hospital versus community acquisition after date of admission underestimates the proportion of infections that are community-acquired (the cut-off should be five or six days). Screening of admissions is unlikely to effectively reduce importation of infections from the community because many exposed individuals will be missed by current screening approaches. Infections in hospital are still epidemiologically important because hospitals have a high concentration of susceptible individuals (who have multiple comorbidities and a high exposure to antibiotics and other drugs), and a higher proportion of individuals are discharged from hospitals carrying *C. difficile* than are admitted. Improving hygiene and sanitation, and reducing average length of stay (as opposed to antibiotic stewardship) are the most cost-effective means of reducing infection rates in the hospital, particularly when delivered in combination. In the community, infants and animals likely contribute significantly to transmission. Targeted, risk-based surveillance (both within hospitals and at the national level) are supported by the evidence. Such a risk-based approach would consider history of antibiotic and other pharmaceutical drug exposure, comorbidities, time of year and international travel history, and would use evidence-based surveillance definitions that attribute a much higher percentage of hospital-identified cases as being community-acquired than is currently the case. Overall, there needs to be a shift towards a more public health oriented approach to *C. difficile* and other healthcare-acquired infections that has a greater focus on community prevention and away from the current, dominant biomedical approach that focusses on therapeutic solutions.

Oral Abstracts

5

Getting One Health into practice in Fiji

Simon Reid[1], **Mike Kama**[2]

1. The University of Queensland, Herston, QLD, Australia

2. Fiji Centre for Communicable Disease Control, Ministry of Health and Medical Services, ., Fiji

Fiji is developing a multisectoral framework for management of public health hazards at the human-animal-ecosystem interface using a One Health approach. They genesis of this activity was a process to develop a national strategy to control leptospirosis that began in 2011 and finished in 2015. Leptospirosis is a significant cause of morbidity and mortality in the country with approximately 420 laboratory-confirmed cases and 32 deaths per year attributed to the disease. The leptospirosis strategy was then used as a starting point for a broader consultation to address gaps in capacity to meet the requirements of the International Health Regulations in terms of intersectoral collaboration and coordination.

The main conclusion from the consultation process is that there is a need for a process to develop a culture of collaboration at the operational level as well as a governance structure that facilitates/enables collaboration and appropriate resource allocation. To achieve this a number of multisectoral pilot projects were developed on bovine tuberculosis (human exposure), fish poisoning (including Ciguatera), antimicrobial usage and residues in livestock and leptospirosis surveillance. The outcomes of these projects (due for completion in the coming months) will help inform approaches to governance across the implementing agencies in each sector. In the interim the Ministry of Health and Medical Services has endorsed a higher level approach to coordination and created a new governance structure to enhance communication between the different sectors involved in the management of zoonotic diseases under a new national communicable Disease committee.

6

Towards a one health approach: dissemination of antibiotic resistant microbial communities and genes from hospital, municipal wastewater to downstream environments

Barbara Drigo[1], **Jan Bell**[2], **Gianluca Brunetti**[1], **Xiayuan Wu**[1], **Samuel Aleer**[1], **Michael Short**[1], **Rietie Venter**[3], **Enzo Lombi**[1], **Erica Donner**[1]

1. Future Industries Institute, University of South Australia, Adelaide, SA, Australia

2. The University of Adelaide, Adelaide, SA, Australia

3. School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

Municipal wastewater is emerging as an ideal composite matrix for monitoring human and environmental phenomena on a community scale. Systematic analysis of municipal wastewater has already proven useful as a means of providing population-scale information on human exposure to chemicals such as licit and illicit drugs. While very much an emerging field, wastewater monitoring could in future be envisaged to deliver valuable population health outcomes such as facilitating early-onset warning of infectious disease outbreaks. Here, we monitored the emergence of antibiotic resistance and opportunistic human pathogens by sampling wastewater from hospitalized, healthy populations, wastewater treatment plants and reclaimed water in South Australia. We used a combination of DNA based high throughput sequencing and quantitative methods (digital droplet PCR and qPCR), and phenotypic screening of opportunistic pathogens and antibiotic resistant bacteria, fungi and protozoa. Results showed that important resistance genes such as *NDM, oxa-48, VIM, SME* and *qnrS* were significantly more abundant in municipal wastewater treatment influents than in hospital-specific wastewater sources. Targeted resistance gene analysis indicated that problematic and emerging resistance threats, such as methicillin resistant *Staphylococcus aureus*, KPC-producing bacteria, and plasmid-mediated resistance to colistin are already widespread in community wastewater, and in some cases putatively more prevalent in the general community than in hospitalized patients. Recycled water entering the reuse pipelines was of demonstrably high quality with *IntI-1*, *blaTEM*, *vanA*, *qnrS*, *sul1* and *ctx-m 32* all below detection by standard qPCR analysis, however digital droplet PCR analysis was sensitive enough to detect residual antibiotic resistant genes and to follow their regrowth potential. This research gives insight into the opportunities and complexities of using municipal wastewater for microbial risk surveillance.

7

The gut microbiota shapes mucosal inflammatory tone and homeostasis in inflammatory bowel disease.

Rabina Giri[1], **Sian Pottenger**[2], **Mark Morrison**[2], **Jakob Begun**[1], **Paraic O Cuiv**[2]

1. Mater Research Institute-The University of Queensland, Woolloongabba, QLD, Australia

2. The University of Queensland Diamantina Institute, Translational Research Institute, University of Queensland, Woolloongabba, QLD

Inflammatory Bowel Disease (IBD) is an incurable disease characterised by episodic and disabling inflammation of the gut. The healthy gut microbiota regulates the tenor of host intestinal mucosal immunity and predisposition to inflammation. With that context, the healthy and IBD gut microbiota differ – notably, the IBD microbiome is characterised by a state of “dysbiosis” with a reduction in the abundance of key Firmicutes taxa affiliated with *Clostridium* cluster IV and XIVa, and an expansion of pro-inflammatory pathobionts. The gut microbiota has co-evolved with the mucosal immune system raising the intriguing possibility that it fulfils a key ecological role as an extrinsic regulator of inflammatory tone. To explore this possibility, we used an innovative approach termed metaparental mating to selectively isolate genetically tractable Firmicutes affiliated bacteria and assessed the ability of 24 isolates to suppress NF-κB activation using our LS174T and Caco-2 gut epithelial reporter cell lines. NF-κB is a master regulator of inflammation and is central to the pathogenesis of IBD. From this screen we identified *Enterococcus faecalis* AHG0090 and strains affiliated with *Clostridium*cluster XIVa (AHG0001, AHG0002, AHG0011) and XV (AHG0017) that produced potent low molecular weight NF-κB suppressive bioactives (Z-score <-3). Critically, none of the suppressive strains exhibited cytotoxic activity. NF-κB suppression was affected by culture conditions and there were dramatic intraspecies variations in suppressive capacity. The bioactives could be broadly separated into two classes possibly inclusive of both peptides and small molecules: The first class are heat and proteinase K labile (e.g. AHG0011 and AHG0090) while the second class are resistant (e.g. AHG0001, AHG0002 and AHG0017). The isolates suppressed NF-κB-p65 subunit nuclear translocation in Caco-2 cells and pro-inflammatory IL-8 secretion in peripheral blood mononuclear cells and gut epithelial organoids from healthy and IBD subjects. Our findings show the extent of microbe-derived NF-κB suppressive

capacity is more common than previously appreciated, and a loss of extrinsic NF-κB regulatory capacity from the gut ecosystem may contribute to IBD risk and recurrence.

1. Ó Cuív, P., Giri, R., Hoedt, E. C., McGuckin, M. A., Begun, J. and Morrison, M. Enterococcus faecalis AHG0090 is a genetically tractable bacterium that produces a secreted peptidic bioactive and suppresses NF-kB activation in human gut epithelial cells. Frontiers in Immunology. https://doi.org/10.3389/fimmu.2018.00790

8

Virulence characteristics of adherent-invasive *Escherichia coli* isolated from healthy individuals, patients and the environment

Mohammad Katouli[1], Dylan Astley[1], Nicole Masters[1], Anna Kuballa[1]

1. University Of The Sunshine Coast, Sippy Downs, QLD, Australia

Collectively, ulcerative colitis (UC) and Crohn's disease (CD) constitute idiopathic inflammatory bowel disease (IBD). While the pathogenic mechanisms underlying IBD remain poorly characterized, *Escherichia coli* has been implicated as a microbiological factor in disease pathogenesis. Increased numbers of mucosa-associated *E. coli* have consistently been identified in the gut of patients with IBD and colorectal cancer (CRC). Designated as adherent-invasive *E. coli* (AIEC), these strains show an enhanced ability to diffusely adhere (DA) to-, and invade intestinal epithelial cells (IECs), along with the ability to survive and replicate within macrophages. It has been shown that AIEC strains harbor specific virulence genes (VGs) associated with their pathogenicity. We investigated the presence of *E. coli* clones carrying phenotypic and genotypic traits consistent with AIEC among 808 diffusely adherent *E. coli* strains isolated from healthy individuals (HI), patients with community-acquired (CA) UTI, hospitalized patients with septicemia or urosepsis, sewage treatment plants (STPs) and surface waters (SW). Typing of the isolates, including phylogenetic grouping showed that they belonged to 48 common clones (CCs). Representatives of each of the CC were tested for their ability to invade Caco-2 cells, survive and replicate within macrophages, and the presence of six AIEC-associated VGs. Thirty-three percent of the isolates, belonging to 20 CCs, showed the ability to survive and replicate within macrophages, whilst containing the genes *dsb*A, *htr*A and *clb*A. These strains were sourced primarily from HI and CA-UTI patients (7 CCs each) and STPs (4 CCs). CA-UTI strains showed a significantly ($P<0.001$) higher intracellular bacterial load (6,929 ± 557 c.f.u. well-1) than others. Two CCs of the AIEC from CA-UTI patients and HI (one each) were found in five out of the six sources investigated. High presence of AIEC strains found in the gut of HI, not only implies their involvement in pathogenesis of CA-UTI, but also suggests the survival of these strains in STPs and the environment.

9

Targeting the middle man: *Staphylococcus aureus* targets human dendritic cells thereby hindering the activation of effector T lymphocytes

Victor Torres[1]

1. NYU School of Medicine, New York, NEW YORK, United States

Staphylococcus aureus is a human pathogen responsible for high morbidity and mortality worldwide. Recurrent infections with this bacterium are common, suggesting that *S. aureus* thwarts the development of sterilizing immunity. Strains that cause disease in humans produce an array of bi-component, pore-forming toxins, commonly known as leukocidins, that target and injure immune cells. However, the contribution of these toxins to the observed impairment of adaptive immunity towards *S. aureus* is not fully understood. Dendritic cells (DCs) are professional antigen-presenting cells required for the training and development of adaptive immunity. I will discuss our recent findings on the effects of leukocidins on human DCs. By taking advantage of an *ex vivo* infection model of primary human monocyte-derived dendritic cells, we found that *S. aureus*, including strains from different lineages and drug resistance profiles, efficiently targets and kills human DCs. We observed that although *S. aureus* is taken up readily by DCs, the bacteria are nevertheless very potent at killing these cells. Infections with live bacteria lacking each of the individual toxins revealed that *S. aureus*-mediated killing of DCs is driven by a single leukocidin known as LukAB. Experiments using co-culture experiments with DCs, *S. aureus*, and autologous CD4+ T lymphocytes further unraveled that by targeting human DCs, LukAB inhibits DC-mediated activation and proliferation of primary human T cells. Altogether, these findings reveal a novel immunosuppressive strategy of *S. aureus*, whereby the bacterium can blunt the development of adaptive immunity via LukAB-mediated injury of DCs.

10

Spore Wars: strategies combating spore-forming pathogens

Yogitha N Srikhanta[1], Melanie L Hutton[1], Milena M Awad[1], Nyssa Drinkwater[1], Julie Singleton[1], Bliss A Cunningham[1], Sheena McGowan[1], Dena Lyras[1]

1. Monash University, Clayton, VIC, Australia

Spore-forming bacteria encompass a diverse range of genera and species, including important human pathogens. Due to their inherent resistance and unique structure, spores are highly problematic in health care settings, in the food industry and as potential bioterrorism agents, resulting in high economic costs and disease burden. *Clostridium difficile* is a spore-forming pathogen and global health threat, responsible for outbreaks of hospital-acquired diarrhoea. Even though spores are critical in *C. difficile* disease transmission and recurrence, current treatments are ineffective against spores. We identified a group of compounds (DL01, DL02, DL03) which have inhibitory effects on *C. difficile* sporulation *in vitro* and *in vivo*. Excitingly, we found that co-treatment of mice with DL02 and the current standard-of-care, vancomycin, prevented disease recurrence. TEM imaging suggests that these compounds block early stages of sporulation. Through mass spectrometry, mutagenesis and binding studies, we determined that the compounds target spore-specific proteins. This family of compounds directly impacting on sporulation could contribute to current regimes in treating *C. difficile* infections. Importantly, this anti-sporulation study could significantly advance drug development for other important spore-forming pathogens.

Oral Abstracts

11

Structural insights into the regulation and inhibition of bacterial autotransporter proteins

Jason J Paxman[1], Alvin Lo[2], Tony Wang[1], Mark Schembri[2], Begoña Heras[1]

1. Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, Australia

2. School of Chemistry and Molecular Biosciences , The University of Queensland, Brisbane, QLD 4072, Australia

The autotransporter family is the largest group of secreted and outer-membrane proteins in Gram-negative bacteria. These proteins perform a vast array of functions linked to pathogenesis, from adhesion and invasion of human host cells to the formation of cell aggregates and biofilms on biotic and abiotic surfaces. The self-associating autotransporters (SAATs) are a sub-group of autotranporters widespread across pathogens [1]. These proteins promote the formation of aggregated communities and biofilms, which facilitate host colonization and bacterial persistence in different environmental niches.

We previously elucidated the mechanism by which the SAAT Antigen43 from uropathogenic *E. coli* (UPEC) promotes bacterial aggregation/biofilm formation, by means of self-association between neighbouring cells [2]. We sought to determine if all SAATs shared a common mechanism for facilitating bacterial aggregation/biofilm formation, if this function was regulated and if it could be inhibited. TibA is a multifunctional SAAT from enterotoxigenic *E. coli* (ETEC), the leading bacterial cause of diarrhea. This surface protein was known to be glycosylated by the cognate glycosyltransferase TibC. We determined the crystal structures of the glycosylated and unglycosylated forms of TibA and used this to inform further biophysical and phenotypic studies. We found that TibA self-associates in a head-to-tail manner with an extensive interface, to facilitate bacterial aggregation/biofilm formation. Glycosylation by TibC was found to physically block TibA self-association to reduce bacterial aggregation/biofilm formation. Our comprehensive structural and functional analysis provide a molecular understanding of how a post-translational modification switches the activity of TibA from an aggregative molecule to and adhesin and invasin. This may represent a general mechanism for bacteria to regulate the virulence functions of the vast number of SAAT expressed on their cell surface.

We have also developed a nM inhibitor of SAAT mediated aggregation/biofilm formation and have determined the first autotransporter-inhibitor crystal structure. Molecules that block bacterial cell clusters and biofilms could be used in synergy with antibiotics, detergents or anti-biofilm agents to improve their efficacy, which would impact environmental, industrial, and human medical microbiology.

1. Vo JL, Martínez Ortiz GC, Subedi P, Keerthikumar S, Mathivanan S, Paxman JJ# and Heras B#. Autotransporter adhesins in Escherichia coli pathogenesis. Proteomics 2017 Jun 30. doi: 10.1002/pmic.201600431.

2. Heras B, Totsika M, Peters KM, Paxman JJ, Gee CL, Jarrott RJ, Perugini MA, Whitten AE and Schembri MA (2014) The antigen 43 structure reveals a molecular Velcro-like mechanism of autotransporter-mediated bacterial clumping, Proc Natl Acad Sci USA 111, 457-462.

12

Genome-wide discovery of genes required for capsule production by uropathogenic *Escherichia coli*

Kelvin G.K Goh[1], Minh Duy Phan[1], Brian M Forde[1], Glen C Ulett, Matt J Sweet[2], Scott A Beatson[1], Mark A Schembri[1]

1. School of Chemistry & Molecular Biosciences, and Australian Infectious Diseases Research Centre , University of Queensland, Brisbane, Queensland, Australia

2. Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia

Uropathogenic *Escherichia coli* (UPEC) are a major cause of urinary tract and bloodstream infections, and possess an array of virulence factors for colonization, survival and persistence. One such factor is the polysaccharide K capsule. Among the different K capsule types, the K1 serotype is strongly associated with UPEC infection. In this study, we sequenced the genome of the K1 UPEC urosepsis strain PA45B, and employed a novel combination of a lytic K1 capsule-specific phage, saturated Tn*5* transposon mutagenesis, and high-throughput transposon directed insertion-site sequencing (TraDIS) to identify the complement of genes required for capsule production. Our analysis identified known genes involved in capsule biosynthesis, as well as two additional regulatory genes (*mprA* and *lrhA*) that we characterized at the molecular level. Mutation of *mprA* resulted in protection against K1 phage-mediated killing, a phenotype restored by complementation. We also identified a significantly increased Tn*5* insertion frequency upstream of the *lrhA* gene, and showed that strong expression of LrhA led to loss of capsule production. Further analysis revealed mutation of *mprA* or overexpression of LrhA resulted in decreased transcription of capsule biosynthesis genes in PA45B, and increased sensitivity to killing in whole blood. Similar phenotypes were also observed in other UPEC strains UTI89 (K1) and CFT073 (K2) when *mprA* was mutated or LrhA was overexpressed, demonstrating that the effects were neither strain nor capsule type specific. Overall, this study defined the genome of a UPEC urosepsis isolate, and identified and characterized two new regulatory factors that affect UPEC capsule production.

13

Complexity of interactions between uropathogenic *E. coli* strains and the innate immune system: role of the hemolysin A toxin and the cof phosphatase in initiating human macrophage cell death

Ambika Murthy[1], Kolja Schaale[1], Matthew Sullivan[2], Alvin Lo[3], Nhu Nguyen[3], Glen Ulett[2], Mark Schembri[3], Matt Sweet[1]

1. Institute for Molecular Bioscience and the Australian Infectious Diseases Research Centre, The University of Queensland, , QLD 4072

2. School of Medical Science, and Menzies Health Institute , Griffith University, Gold Coast, Queensland, Australia

3. School of Chemistry and Molecular Biosciences, Brisbane

Uropathogenic *E. coli* (UPEC) causes the majority of urinary tract infections (UTI), which is associated with significant morbidity and mortality. An understanding of the interactions between UPEC and the innate immune system may lead to new therapeutic approaches for UTI. We recently demonstrated that some UPEC strains rapidly kill human macrophages, likely as a host evasion mechanism. Using a random transposon mutagenesis library in the reference strain CFT073, we identified eight clones that were impaired in their ability to kill primary human macrophages. These hits were

independently validated and sequenced to identify mutated genes, and independent knock-outs were generated to further validate the findings. This approach revealed that the pore forming toxin, hemolysin A (HlyA) is essential for triggering both cell death and inflammasome-dependent IL-1β release in human macrophages. The poorly characterized Cof phosphatase was also identified as a novel hemolysin regulator. In surveying isolates of the clinically-relevant globally disseminated *E. coli* sequence type 131 (ST131) clone, we also found that only HlyA$^+$ strains kill human macrophages. Further characterization of these strains revealed variation in the levels of HlyA secretion, and consequent heterogeneity in their capacity to trigger human macrophage cell death. This impacted on mechanisms of cell death; in an ST131 strain that secretes low HlyA levels, cell death was partially dependent on the NLRP3 inflammasome. In contrast, in a UPEC strain secreting high amounts of HlyA, cell death was NLRP3 independent. To understand the functional relevance of this, the effect of HlyA levels on bladder colonization was assessed in a mouse UTI model. These studies revealed that fine-tuning of HlyA production likely dictates host evasion verses host protection. Overall, this study highlights that HlyA mediates UPEC-initiated inflammasome activation and cell death in human macrophages, and that an NLRP3-independent cell death pathway exists in these cells. We are currently investigating the alternative cell death pathway, as this likely has important implications for understanding UTI.

14

Identification of *Pasteurella multocida* small RNAs: unravelling the regulatory network

Marianne N Mégroz[1], Emily Gulliver[1], Brandon Sy[2], Julia Wong[2], David Powell[3], Adele Barugahare [3], Jai J Tree[2], Marina Harper[1], John D Boyce[1]

1. Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University , Clayton, Victoria, Australia

2. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia

3. Monash Bioinformatics Platform, Monash University, Melbourne, VIC, Australia

The Gram-negative bacterium *Pasteurella multocida* is the causative agent of a number of economically important animal diseases, including avian fowl cholera. Numerous *P. multocida* virulence factors have been identified, including capsule, lipopolysaccharide (LPS) and filamentous hemagglutinin, but little is known about how the expression of these virulence factors is regulated. Small non-coding RNA molecules (sRNAs) are important regulators of bacterial gene expression and protein production, with essential roles in controlling diverse bacterial functions including virulence. Hfq is an RNA-binding protein that facilitates riboregulation via interaction with small noncoding RNA molecules (sRNAs) and their mRNA targets. A *P. multocida hfq* mutant produced significantly less hyaluronic acid capsule during all growth phases and displayed reduced *in vivo* fitness compared with the wild-type strain. The *hfq* mutant also displayed global changes in gene expression and protein production, including altered expression of the key *P. multocida* virulence factors, capsule, filamentous hemagglutinin and LPS. These data indicate that Hfq and associated sRNAs are likely to be critical regulators of *P. multocida* virulence. To further examine sRNA-mediated riboregulation in *P. multocida*, putative *trans*-encoded sRNAs were identified using a combination of bioinformatics, RNA-Seq, Hfq co-immunoprecipitation, Hfq UV–crosslinking and analysis of cDNA (Hfq-CRAC) and Hfq UV-crosslinking, ligation and sequencing of hybrids (Hfq-CLASH) experiments. These combined analyses identified more than 50 putative sRNAs; most were encoded in intergenic regions, highly expressed, and contained putative Rho-independent terminators. A number of these putative sRNAs were differentially expressed under specific conditions, such as growth in low-iron medium or under anaerobic conditions, suggesting that they may have roles in regulating global responses to these conditions. Finally, the Hfq co-immunoprecipitation, Hfq-CRAC and Hfq-CLASH experiments define the global set of sRNA and mRNA interaction sites. Twenty Hfq-associated hybrids were identified using Hfq-CLASH, including *hfq* and one sRNA. A number of putative sRNAs have been selected for experimental validation and detailed functional characterization.

15

The metabolic potential of ASGARD archaea in light of eukaryogenesis

Anja Spang[1, 2], Courtney Stairs[1], Nina Dombrowski[3], Laura Eme[1], Jonathan Lombard[1], Eva Fernandez Caceres[1], Steffen L. Jorgensen[4], Brett J. Baker[2], Thijs J.G. Ettema[1]

1. Royal Netherlands Institute for Sea Research, AB Den Burg (Texel), Netherlands

2. Department of Cell- and Molecular Biology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

3. Department of Marine Science, University of Texas-Austin, Marine Science Institute, Port Aransas, United States

4. Department of Biology, Centre for Geobiology, University of Bergen, Bergen, Norway

The origin of eukaryotes represents an unresolved puzzle in evolutionary biology. For a long time, it was assumed that Archaea have played a central role in hypotheses on eukaryogenesis, and recent findings support the view that eukaryotes evolved from a symbiosis between an archaeal host and an alphaproteobacterial endosymbiont. The recent discovery of the Asgard superphylum, has shed additional light on this enigmatic event. Asgard archaea, whose genomes were obtained by metagenomics from various sediment samples across the world, represent the closest prokaryotic relatives of eukaryotes identified so far. Furthermore, their genomes harbor a plethora of eukaryotic signature proteins that may have been key in the evolution of complex eukaryotic cells. So far however, little is known about their metabolic potential and the evolution of their functional characteristics.

To unveil the metabolism of the elusive archaeal ancestor of eukaryotes, we have reconstructed the metabolism of Asgard archaea in a comparative genomics framework. Our analyses suggest, that the four different Asgard phyla, i.e. Odin-, Thor-, Loki- and Heimdallarchaeota are metabolically diverse and characterized by different metabolic lifestyles. While Thor- and Lokiarchaeota seem to be able to fix carbon via the Wood-Ljungdahl pathway and may be able to obtain energy from various organic substrates including fatty acids and perhaps hydrocarbons, Heimdallarchaeota seemingly encode terminal oxidases suggesting the ability to reduce nitrate or even oxygen. In contrast, Odinarchaeum might be a fermentative organism likely able to use H+ as electron acceptor. Altogether, our current analyses of these metabolic features and the reconstruction of the evolution of selected metabolic traits in the diversification of this superphylum show that previous inferences on the nature of the archaeal ancestor of eukaryotes need to be reconsidered and provide a more comprehensive basis for the formulation of an updated scenario on the evolution of eukaryotes from a metabolic point of view.

16

After the storm: Microbial community dynamics and antibiotic resistance within an anthropogenically impacted urban beach

Justin Seymour[1], Richard Carney[1], Nahshon Siboni[1], Mark Brown[2], Maurizio Labbate[1]

1. University Of Technology, Sydney, BROADWAY, NSW, Australia

2. University of Newcastle, Newcastle, NSW, Australia

Coastal ecosystems represent a highly dynamic interface for human interaction with environmental microbial assemblages. We examined the dynamics of a coastal microbial community inhabiting a highly impacted urban beach, using a high resolution (weekly), two-year duration time-series. Microbial assemblage structure was characterised using 16S rRNA amplicon sequencing and several pathogens and antibiotic resistance genes were targeted using qPCR. Microbial community composition was highly dynamic, with patterns often reflective of anthropogenic impacts. Specifically, several Operational Taxonomic Units (OTUs) exhibited pronounced peaks in relative abundance that exhibited both direct, and time-lagged, correlations to stormwater and sewage inputs. Among these were OTUs from the Arcobacter genus, a group including pathogens often associated with sewage and wastewater infrastructure. Arcobacter relative abundance regularly increased from < 1% to > 70% of sequences within 1 week, with network analysis revealing links with stormwater and sewage. These anthropogenic impacts also led to 1-2 order of magnitude increases in the abundance of several antibiotic resistance genes, including genes conferring resistance to both high use front-line and last resort antibiotics. Network analysis linked the occurrence of these genes to Arcobacter and several other putative pathogens. Finally, frequent peaks in abundance of potentially pathogenic endemic marine microbes, including members of the Vibrio genus, were observed, but rather than being correlated to stormwater or sewage, were linked to marine heat-waves. These observations demonstrate that coastal microbial assemblages are shaped by a range of anthropogenic forces and are characterised by intense peaks in the abundance of potentially pathogenic and antibiotic resistant microbes.

17

Not available at time of printing

Nicole Webster[1]

1. Australian Institute of Marine Science, Townsville, QLD, Australia

Not available at time of printing

18

Vibrio cholerae senses and responds to the presence of the predator, *Tetrahymena pyriformis* by increasing biofilm formation

Shuyang Sun, Martina Erken, Florentin Constancias, Diane McDougald

Protozoan grazing is an important mortality factor of bacteria in aquatic habitats. *Vibrio cholerae*, the etiological agent of cholera and natural inhabitant of brackish and seawater, relies on biofilm formation to defend against predation. Here, we investigated the 'reverse grazer effect' of *V. cholerae* biofilms induced by predation by the ciliate, *Tetrahymena pyriformis*.

V. cholerae was exposed to *T. pyriformis* in artificial seawater and biofilm biomass was determined by confocal laser scanning microscopy and crystal violet staining. Co-cultures were also performed using filter inserts to physically separate *V. cholerae* and *T. pyriformis* to determine if physical contact was required for the increase in biofilm biomass. The nutrients in the cell-free supernatants of *T. pyriformis* were quantified by biochemical assays and LC-MS, and addback experiments were performed to confirm their effects on *V. cholerae* biofilm formation. RNA-Seq and RT-PCR were used to identity *V. cholerae* genes involved in supernatant-induced biofilm formation.

When *V. cholerae* was co-incubated with *T. pyriformis,* the bacterium was eliminated in the planktonic phase, but microcolony numbers and surface coverage of the biofilm increased. The stimulation of biofilm formation was also observed when *T. pyriformis* was physically separated from the bacterial biofilm by filter inserts. The supernatants were analysed and it was determined that phosphate concentration (0.59 mM) and the presence of 4 amino acids (arginine 3.3 μM, asparagine 1.2 μM, glutamine 3.4 μM and serine 2.7 μM) in the *T. pyriformis* supernatant were important for stimulation of the *V. cholerae* biofilms, while carbon and nitrogen were not critical. RNA-Seq revealed that 112 transcripts were up-regulated and 371 transcripts were down-regulated in response to the cell-free supernatant. RT-PCR confirmed that the 4 amino acids induced *V. cholerae* biofilm formation through c*srA*. This data shows that *V. cholerae* has the ability to sense and respond to the presence of a protozoan predator by forming grazing resistant biofilms.

19

Tissue preparation and nucleic acid purification influences the outcome of Pacific oyster (*Crassostrea gigas*) microbiome studies

Erandi Pathirana[1], Richard Whittington[1], Alison Tweedie[1], Paul Hick[1]

1. Farm Animal Health, Sydney School of Veterinary Science, Faculty of Science, The University of Sydney, Camden, NSW, Australia

Pacific oysters harbour a rich bacterial community of which a substantial proportion is non-cultivable. Bacterial DNA extraction in oyster microbiome studies faces many limitations. Different sampling techniques and nucleic acid extraction methods were tested to optimize the bacterial DNA yield and to accurately determine the microbiome associated with different oyster tissues.

Samples of haemolymph, swab samples and tissue samples of gills, gut and adductor-muscle were collected from Pacific oysters grown in the Georges River, NSW (n=10). Selected samples were enriched for bacterial DNA. Nucleic acids were extracted using three different commercial extraction kits.

Oral Abstracts

Bacterial DNA yields were assessed by a real-time PCR assay targeting the 16S rRNA gene. Bacterial community compositions were identified by high-throughput sequencing of the V1-V3 regions of the 16S rRNA gene. The relative abundance of bacterial phyla and diversity of OTUs were calculated for each sample. Beta diversity of bacterial communities obtained after different tissue preparation strategies were analysed.

Phylum *Proteobacteria* dominated in all tissue types except gut tissue where phylum *Fusobacteria* was abundant. *Bacteroidetes*, *Spirochaetes* were among the other abundant phyla. Beta diversity of bacterial community structure in different tissue types were significantly different. Higher bacterial diversity and bacterial DNA yield were noted in gill tissues compared to gut tissues ($p<0.05$). The highest bacterial DNA yield was obtained with the QIAamp® DNA microbiome kit extracts despite the lower bacterial diversity compared to EZNA Mollusc DNA kit extracts ($p<0.05$).

Apart from tissue specificity of the oyster microbiome, the tissue preparation strategy affects the quantity and diversity of bacteria identified in microbiome studies of the Pacific oyster. A clearly defined and fit-for-purpose sample preparation strategy is required to accurately identify the oyster microbiome.

20

Confirmation of anatoxin-a producing cyanobacteria in Australia: evidence of a changing global distribution?

Nijoy John[1, 2], Louise Baker[2], Brendan Ansell[2], Steven Newham[3], Nick Crosbie[4], Aaron Jex[1, 2]

1. Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, Victoria, Australia

2. Population Health and Immunity, Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

3. Goulburn Valley Water, Shepparton, Victoria, Australia

4. Melbourne Water, Docklands, Victoria, Australia

Toxic cyanobacterial blooms pose significant public health and water quality risks in surface waters worldwide.Changes in global climate and rainfall patterns have led to increased frequency of cyanobacterial blooms, rapid shifts in the distributions of many cyanobacteria species and emergence of novel toxin types in previously toxin-free regions. Our study investigated the emergence of anatoxin-a (ATX-a) producing cyanobacteria in Australian surface waters (a continent previously assessed as being anatoxin-free) using microscopy, nested Polymerase Chain Reaction (PCR)-based amplification of the ATX-a synthetase gene and direct detection of ATX-a by Enzyme-Linked Immunosorbent Assay (ELISA). Screening samples were collected over a period of seven years across the state of Victoria, Australia. Here, we (1) detected ATX-a producing cyanobacteria, including *Cuspidothrix issatschenkoi*, *Aphanizomenonaceae*, *Dolichospermum* sp. and *Oscillatoria* sp., from 31 different sampling locations, (2) identified the presence and distribution of cyanobacteria encoding the *ana*C gene required for ATX-a production, and (3) determined the presence and concentration of ATX-a in our samples. Our study highlights the importance of regular investigation to monitor emerging and shifting distributions of toxic cyanobacteria worldwide in a period of increased eutrophication and rising surface water temperatures.

21

Targeting membrane transporters of the malaria parasite with antitubercular drugs

Natalie J Spillman[1], Daniel E Goldberg[2]

1. School of BioSciences, Faculty of Science, University of Melbourne, Melbourne, VIC, Australia

2. Washington University in Saint Louis School of Medicine, Saint Louis, MO, USA

The identification of new antimalarial compounds is critical, as the *Plasmodium* parasite has developed resistance to all currently used drugs. One possibility to address this problem is to repurpose compounds with activity against other pathogens. We have characterized two exported epoxide hydrolases (EH) of *Plasmodium falciparum*, capable of hydrolysing bioactive epoxy-fatty acids into less active diols. To identify inhibitors of the parasite EHs we screened a library of adamantyl urea (AU)-based inhibitors, originally designed to target *Mycobacterium tuberculosis (Mtb)* EHs. Although some AU compounds inhibited the growth of malaria parasites, *in vitro* killing did not correlate with *in vitro* EH enzyme inhibition. However, killing of *Plasmodium* parasites correlated with *Mtb* killing. In *Mtb*the AU EH inhibitors have additional targets, with AU-resistant *Mtb* harboring mutations in the lipid transporter, MmpL3 (an antiporter). However, *Plasmodium* has no MmpL3 orthologue. Therefore, to investigate the target in the malaria parasite, we raised parasites that were resistant to the most potent AU inhibitor. Whole genome sequencing of two-independent selections revealed non-synonymous mutations in a different lipid transporter- a class 4, P-type lipid-transporting ATPase. Creation of a transgenic line overexpressing the ATPase confirmed that this protein confers parasite resistance to the AU inhibitor. The antimalarial action of another MmpL3 inhibitor (SQ109) was also investigated in *Plasmodium* parasites, with another parasite membrane transporter implicated in the mechanism of action/resistance. The hydrophobic nature of the antitubercular compounds may result in membrane accumulation and could explain why transporters were identified in resistance studies in both *Mtb* and *Plasmodium*.

22

Not available at time of printing

Cynthia Whitchurch[1]

1. The ithree institute, University Of Technology Sydney, Sydney, NSW, Australia

Not available at time of printing

Oral Abstracts

23

Not available at time of printing

Gregory Moseley

Not available at time of printing

24

Green Fluorescent Protein as a tool for studying Group B Streptococcal microbiology

Matthew J Sullivan[1], Glen C Ulett[1]

1. School of Medical Science and Menzies Health Institute Queensland, Griffith University, Gold Coast, Queensland

Group B Streptococcus (GBS; *Streptococcus agalactiae*) is a globally disseminated opportunistic pathogen that causes meningitis, sepsis, pneumonia and soft tissue infections in neonates and healthy or immunocompromised adults (*1-4*). GBS is the leading cause of neonatal mortality due to bacterial infection, and in developing countries up to 3 babies per 1000 live births develop GBS infection, with a fatality rate as high as 15% (*1, 2*). GBS is also a prominent veterinary pathogen of bovine mastitis (*5*), and extensive use of tetracycline facilitated transmission of distinct clones of GBS into humans (*6*). Many studies have examined GBS physiology, virulence and microbe-host interactions using diverse imaging approaches, including fluorescence microscopy. Strategies to label and visualize GBS using fluorescence biomarkers have been primarily limited to antibody-based methods or non-specific stains that bind DNA or protein, and use dead cells and/or tissues.

In this study, we developed and validated a green fluorescence protein (GFP)-expressing plasmid, pGU2664 that can be applied as a marker to visualize GBS in experimental studies. The synthetic, constitutively active CP25 promoter drives strong and stable expression of GFP in GBS carrying pGU2664. GBS maintains GFP activity across different phases of growth. Application of fluorescence polarization enables easy discrimination of GBS GFP activity from the auto-fluorescence of culture media commonly used to grow GBS, such as THB. Differential interference contrast microscopy, in combination with epifluorescence microscopy to detect GFP in GBS, enabled visualization of bacterial attachment to live human epithelial cells in real time. Plasmid pGU2664 was also used to visualise phenotypic differences in the adherence of wild-type GBS and an isogenic gene-deficient mutant strain lacking the control of global virulence regulator CovR in live adhesion assays. The system for GFP-expression in GBS described in this study provides a new tool for the visualization of this organism in diverse research applications.

1. J. W. Larsen, J. L. Sever, Group B Streptococcus and pregnancy: a review. Am. J. Obstet. Gynecol. 198, 440-448; discussion 448-450 (2008).
2. P. J. Steer, J. Plumb, Myth: Group B streptococcal infection in pregnancy: comprehended and conquered. Seminars in fetal & neonatal medicine 16, 254-258 (2011).
3. A. K. Johri et al., Group B Streptococcus: global incidence and vaccine development. Nat. Rev. Microbiol. 4, 932-942 (2006).
4. T. H. Skoff et al., Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. Clin. Infect. Dis. 49, 85-92 (2009).
5. G. Keefe, Update on control of Staphylococcus aureus and Streptococcus agalactiae for management of mastitis. Vet. Clin. North Am. Food Anim. Pract. 28, 203-216 (2012).
6. V. Da Cunha et al., Streptococcus agalactiae clones infecting humans were selected and fixed through the extensive use of tetracycline. Nat. Commun. 5, 4544 (2014).

25

Nanoscale imaging of protein secretion systems used by bacteria and their viruses

Iain D Hay[1], Trevor Lithgow[1]

1. Monash University, Infection & Immunity Program, Biomedicine Discovery Institute & Dept of Microbiology, Clayton, VIC, Australia

Secretins form large (~150 Å), homo-oligomeric, gated pores in the outer membrane (OM) of bacteria. Each secretin complex comprises 12-16 copies of the secretin protein. Indicative of the quintessential nature of this family of proteins, secretins are ubiquitous among all didermic bacterial phyla and also found in some phage genomes. They form the OM component of the Type Four Pili (T4P), the Type Two Secretion System (T2SS), the Type Three Secretion System (T3SS/Injectisome) and are also required for the assembly and export of filamentous phage (Inoviridae).

The T2SS is unique in that it exports a range of soluble folded exoproteins directly from the periplasm, commonly hydrolytic enzymes used to degrade biopolymers (proteins, carbohydrates, lipids) for nutrient acquisition, but also far more nefarious virulence factors such as the Cholera toxin, ETEC Heat-labile enterotoxin, or *Pseudomonas* Exotoxin A. Previously referred to as the terminal component of the general secretion system, it is now apparent that the T2SS is a more specialised secretion machine which must selectively recruit pre-exoproteins from the densely packed periplasm.

Here we present our recent advances in characterising bacterial T2SS secretins. The distribution and classification of these proteins will be discussed and our recent structural insights into the function and assembly of these complex molecules will be provided.

26

Nanoparticles loaded chitosan-polyethylene oxide constructs to combat antimicrobial resistance: A drive towards better performance

Hafiz M. N. Iqbal[1], Angel M. Villalba-Rodríguez[1], Roberto Parra-Saldivar[1]

1. Tecnologico de Monterrey, School of Engineering and Science, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico., Monterrey, MEXICO, Mexico

The antimicrobial resistance (AMR) has become a serious health issue, globally. Finally, after decades of negligence, the AMR issue has now captured the worldwide attention of the global leaders, the public health community, legalization authorities, academia, research-based organizations, and medicinal sector of the modern world, alike. Research is underway around the globe to develop novel strategies and measures to combat AMR issue. Among various approaches, biomaterials-based therapeutic constructs are of supreme interests due to their unique physiochemical and structural characteristics, tunable properties, ease of use, biocompatibility, biodegradability, non-toxicity, and efficacy [1-3]. Herein, a facile biosynthesis of silver nanoparticles (AgNPs) and AgNPs-loaded chitosan (CST)-polyethylene oxide (PEO)/polyethylene glycol (PEG) constructs with biomedical potentialities is reported. The UV-Vis spectroscopic profile confirmed the synthesis of AgNPs using methanolic leaves extract of *Taraxacum officinale*. The newly developed AgNPs were characterized using various analytical and imaging techniques including UV-Vis and FT-IR spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS), atomic force microscopy (AFM), and transmission electron microscopy (TEM). The optimally yielded AgNPs at 24 h reaction period were loaded onto various CST-PEO/PEG constructs. A maximum of 93% and 82% loading efficiency (LE) was recorded for CST: PEO/PEG ratios at 2:1 and 2:2, respectively. The anti-bacterial activities of AgNPs-CST-PEO/PEG constructs were tested against Gram-positive and Gram-negative bacterial strains including *Staphylococcus aureus*, *Escherichia coli*, and *Haemophilus influenzae*. As compared to the initial bacterial count, i.e., 1.5×10^8 CFU/mL (control value), AgNPs-CST-PEO/PEG constructs showed a remarkable reduction in the log values. The cytotoxicity profile revealed complete biocompatibility against L929 cell line. However, AgNPs-CST-PEO/PEG constructs showed considerable cytotoxicity up to certain extent against human epithelial cells (HeLa) cancer cells. In conclusion, the highest antibacterial activities along with anti-cancer behavior both suggest the biomedical potentialities of newly engineered AgNPs and AgNPs-loaded CST-PEO/PEG constructs. The engineered constructs may also be potential candidates as NPs or materials-based vaccines for antibacterial vaccination.

Keywords: Antimicrobial resistance, *Taraxacum officinale*, AgNPs, Chitosan, PEO/PEG, Antibacterial, Anticancer

1. [1] Iqbal, H. M. N., Kyazze, G., Locke, I. C., Tron, T., & Keshavarz, T. (2015). In situ development of self-defensive antibacterial biomaterials: phenol-g-keratin-EC based bio-composites with characteristics for biomedical applications. Green Chemistry, 17(7), 3858-3869.
2. [2] Bilal, M., Rasheed, T., Iqbal, H. M. N., Hu, H., Wang, W., & Zhang, X. (2017). Macromolecular agents with antimicrobial potentialities: A drive to combat antimicrobial resistance. International Journal of Biological Macromolecules, 103, 554-574.
3. [3] Bilal, M., Rasheed, T., Iqbal, H. M. N., Li, C., Hu, H., & Zhang, X. (2017). Development of silver nanoparticles loaded chitosan-alginate constructs with biomedical potentialities. International Journal of Biological Macromolecules, 105, 393-400.

27

Cost-effective, Clinically-relevant work up of respiratory and wound specimens

Susan Sharp[1]

1. Kaiser Permanente, Portland, OREGON, United States

Clinical microbiologists are continually challenged to integrate the concepts of cost-effectiveness and clinical relevance into their daily practice. However, many microbiologists find this a daunting task and may be unsure where to begin. Two of the most difficult specimens to deal with in the microbiology laboratory are respiratory and wound specimens. This is because they contain resident flora mixed in with the pathogens causing the infections. It can often be difficult to determine what might be a potential pathogen versus the normal resident flora. This lecture will review two systematic approaches to help the bacteriologist to determine which organisms might be potential pathogens in these culture types. The Q-score and the Q234 systems will both be presented with examples of how each can be used, how they are similar and how they differ. At the conclusion of the presentation the audience should be able to decide which method would fit into their workflow for the work up of respiratory and wound specimens and provide consistent and relevant results to clinicians for care of their patients.

28

The Role of Glycan Interactions in the Cell and Host Tropism of Bacterial Pathogens

Michael Jennings[1]

1. Griffith University, GOLD COAST, QLD, Australia

Many important interactions between bacterial pathogens and their hosts are highly specific binding events that involve host or pathogen carbohydrate structures called glycans. Bacterial adhesins and toxins can exploit host glycans as targets. Host lectins may recognise bacterial glycans in innate immune processes. The molecular details of many bacterial - host interactions remain to be discovered. Understanding these processes is key for the development of novel strategies for the prevention and treatment of disease. In recent years new, high-throughput glycomic technologies have identified new bacterial-host glycointeractions. This presentation will highlight these recent technological advances and research findings on the glycoscience of bacterial pathogenesis.

Oral Abstracts

29

Elucidating the Scs redox pathway and its role in copper tolerance in *Salmonella*

Pramod Subedi, Jason Paxman, Tony Wang, Ashwinie Ukuwela, Zhiguang Xiao, Begoña Heras

Bacteria use disulfide bond (Dsb) forming enzymes to produce functional virulence factors. In addition to the classic Dsb system, which includes an oxidative DsbA/DsbB pathway and an isomerase DsbC/DsbD pathway, genomic analysis of the human pathogen *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium), revealed a second Dsb-like system encoded by the scsABCD locus. This Scs locus contains genes encoding for Scs(A-D) proteins that have been associated with an increased tolerance to copper. However, their mechanistic roles in copper transport and homeostasis in *Salmonella* are still not clearly understood.

To gain a better understanding of the Scs system in *S*. Typhimurium, we carried out a detailed biochemical and biophysical characterisation of ScsB, an inner membrane protein, and ScsC, a periplasmic soluble protein. The results show that ScsB and ScsC work as a redox pair and form an additional Dsb-like reducing pathway. Furthermore, we have also dissected the molecular mechanism underlying Scs proteins mediated copper tolerance in *Salmonella*. Our results demonstrate that both ScsB and ScsC, can bind copper (I) with significant affinities and transfer it to the periplasmic copper binding protein, CueP.

Taken together, we show how the Dsb-like Scs system has evolved to protect against copper toxicity by sequestering and transferring copper to periplasmic copper binding proteins. Our findings in *S*. Typhimurium could have implications on establishing how Gram-negative pathogens that contain similar Dsb-like redox enzymes, deal with the antibacterial action of copper and contribute to bacterial virulence within the host.

1. Pontel, L. B. and F. C. Soncini (2009). Molecular Microbiology 73(2): 212-225.
2. Majowicz, S. E., et al. (2010). Clinical Infectious Diseases 50(6): 882-889.
3. Gupta SD., et al. (1997). Journal of Bacteriology 179: 4977-4984
4. Shepherd M., et al. (2013). Antioxidants & Redox Signaling 19: 1494-1506

30

Cryptococcus and the Swiss Army Knife of Virulence

Kenya E Fernandes[1], Adam Brockway[1], Miriam Haverkamp[2], Christina Cuomo[3], Floris van Ogtrop[1], John Perfect[4], Dee A Carter[1]

1. University of Sydney, Sydney, NSW, Australia

2. Faculty of Medicine, University of Botswana, Gaborone, Botswana

3. Broad Institute, MIT and Harvard University, Cambridge, USA

4. Duke University School of Medicine, Durham, USA

Fungal pathogens *Cryptococcus neoformans* and the *Cryptococcus gattii* complex are responsible for hundreds of thousands of annual deaths. *Cryptococcus* is an encapsulated yeast, and during infection cells have the capacity for substantial phenotypic variation including capsule enlargement, the shedding of capsule, and variations in cell size. In order to examine associations between morphological variation and clinical outcome, we examined a collection of 70 clinical isolates of *C. neoformans* (n=53) and *C. tetragattii* (n=17) recently taken from HIV/AIDS patients with cryptococcal meningitis in Botswana with accompanying clinical data. Isolates were cultured under conditions that simulate stresses encountered *in vivo* (DMEM, 5% CO_2, 37 °C) that are known to induce capsule production and cell size changes. Cells were counterstained with India Ink, visualised by microscopy, and phenotypes were scored and analysed for associations with clinical parameters using SPSS and R. Giant cells (> 15 µm) were significantly associated with *C. tetragattii* (p=0.007) while micro cells (< 1 µm) and shed capsule were only seen in *C. neoformans* isolates. When correlated with clinical data, phenotypic variables fell into two distinct groups associated with differing symptoms: the “big” phenotypes of bigger cells, bigger capsules, and giant cells, and the “small” phenotypes of micro cells and shed capsule. “Big” phenotypes were associated with higher CD4 count and were negatively correlated with nausea and vomiting (p=0.004), symptoms associated with increased intracranial pressure, suggesting that they are induced in early stage infection. “Small” phenotypes were associated with lower CD4 count, were negatively correlated with symptoms associated with meningeal inflammation, and were positively correlated with nausea and vomiting (p=0.015), suggesting that they are produced later during infection and may contribute to immune suppression and promote proliferation and dissemination of infecting cells. Strains possessing all three major morphological variants (giant cells, micro cells, and shed capsule) were rare, but strikingly these were associated with patient death (p=0.017). Our data indicate that the capacity to be highly pleomorphic is an important driver of infection and propose that this plasticity provides *Cryptococcus* with a “Swiss army knife” of virulence attributes that pre-adapt it for survival in a changing environment.

31

Surveying *Bacillus cereus sensu lato* in Tasmanian dairy environments and dairy products to inform food safety risk assessments.

Zoe Bartlett[1]

1. University of Tasmania, Hobart

The *Bacillus cereus sensu lato* group consists of spore forming bacteria that survive pasteurisation, and are associated with foodborne illnesses. This study enumerated presumptive *B. cereus s. l.* isolated from Tasmanian dairy environments; assessed their prevalence in ricotta and whole pasteurised milk; and phenotypically characterised dairy and ricotta sample isolates, including virulence markers.

Soil, faeces, milking cups, teats, and bulk raw milk were sampled from six Tasmanian dairies. Presumptive *B. cereus s. l.* was present in all soil (M = 4.5 ± 0.25$\log_{10}$CFU/g), all faecal (M = 3.2 ± 0.11$\log_{10}$CFU/g), 10/12 teat (M = 2.5 ± 0.39$\log_{10}$CFU/teat), and 8/12 milking cup (M = 0.50 ± 0.23$\log_{10}$CFU/milking cup) samples. Six negative bulk raw milk samples from three farms were enriched for 48hrs at 20˚C resulting in 4/6 samples positive for *B. cereus s. l.*.

Five samples of ten brands (n=50) of 1L whole pasteurised milk, and five samples of four brands of ricotta (n=20) were analysed for *B. cereus s. l.* before and after incubation at 25˚C for 48hrs (20˚C for ricotta). Enterotoxin (NHE and HBL) production in milk was also assessed following incubation. Initially 22% of milk samples but no ricotta samples were *B. cereus s. l.* positive, increasing to 88% and 75% respectively following incubation. 88% and 66% of milk samples harboured isolates capable of producing NHE or HBL, respectively.

Phenotypic characterisation of 51 dairy farm isolates identified 8% *B. thuringiensis*, 14% *B. mycoides*, 37% *B. cereus sensu stricto*, and 41% *B. weihenstephanensis*. Among nine ricotta isolates, only *B. cereus s. s.* was identified. NHE was produced in sterile milk by 98% of dairy *B. cereus s. l.* isolates and 100% of ricotta isolates when incubated at 12˚C for 72hrs; and HBL in 3.9% of dairy isolates, and 0% of ricotta *B. cereus s. l.* isolates.

B. cereus s. l. are widely distributed throughout Tasmanian dairy farm environments, and isolates capable of NHE and HBL production are present at low concentrations in bulk raw milk, ricotta, and whole pasteurised milk. However, a high proportion of 1L whole pasteurised milk and 375g ricotta samples contain entero-toxigenic isolates.

32

Folding your way to Greater Pathogenicity; the role of cyclophilins in *Burkholderia pseudomallei* virulence

Nicole M Bzdyl[1, 2], **Kathy Fuller**[2, 3], **Isobel Norville**[4], **Andrew Scott**[4], **Timothy Atkins**[4], **Charlene Kahler**[1, 2], **Tim JJ Inglis**[1, 2, 3], **Mitali Sarkar-Tyson**[1, 2]

1. Marshall Centre of Infectious Disease Research and Training, School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia

2. School of Biomedical Sciences, University of Western Australia, Perth, WA, Australia

3. PathWest Laboratory Medicine, Perth, WA, Australia

4. Microbiology, Dstl, Salisbury, Wiltshire, UK

Burkholderia pseudomallei is the cause of melioidosis, an endemic disease in South-East Asia and Northern Australia, with a mortality rate of ~15%. With rising levels of antibiotic resistance and a lack of effective treatment for melioidosis, novel medical countermeasures are needed. Cyclophilins are enzymes which catalyse the *cis*-to-*trans* isoform change of *xaa*-proline bonds, a rate limiting step in protein folding. Cyclophilins have been associated with virulence in intracellular bacteria. As they are highly conserved, cyclophilins present as anti-virulence targets for many pathogenic bacteria, including *B. pseudomallei*, which encodes two cyclophilin genes, *ppiA* and *ppiB*. This study investigates the role of cyclophilins in *B. pseudomallei* by constructing in-frame deletion mutants; *B.psΔppiA*, *B.psΔppiB* and *B.psΔppiAppiB*.

B.psΔppiA and *B.psΔppiB* were impaired in biofilm formation with *B.psΔppiA* showing a 60% reduction and *B.psΔppiB* showing an 80% reduction in biofilm, suggesting that *B.psΔppiA* and *B.psΔppiB* play different roles in the cell. A 25% decrease in motility was also seen with *B.psΔppiA* and *B.psΔppiB*. *B.psΔppiAppiB* exhibits no motility defect, the reason for this is currently being investigated. Gross morphological changes are also observed by Transmission Electron Microscopy, with *B.psΔppiA* displaying an aberrant outer membrane structure. *B.psΔppiA*, *B.psΔppiB* and *B.psΔppiAppiB* display an increased susceptibility to oxidative stress, reflected in reduced survival in macrophages. *B.psΔppiA* and *B.psΔppiAppiB* show a 50% reduction in bacterial numbers over the initial 9 hours post-infection. *B.psΔppiB* shows greater attenuation in macrophages with initial counts similar to that of *B.psΔppiA*, but intracellular counts decrease over 9 hours. *In vivo* studies using mouse infection models is currently underway to determine the effect of cyclophilins on disease. These results suggest that cyclophilins *ppiA* and *ppiB* play an important role in *B. pseudomallei* virulence.

33

Presence of inhibitory antibodies in patients with *Escherichia coli* urosepsis.

Carrie F Coggon[1], **Andrew Jiang**[1], **Kelvin G Goh**[1], **Ian R Henderson**[2], **Mark A Schembri**[1], **Timothy J Wells**[1]

1. The University of Queensland, Woolloongabba, QUEENSLAND, Australia

2. Institute of Microbiology and Infection, University of Birmingham, Birmingham, United Kingdom

Introduction

Escherichia coli is the most common cause of urinary tract infections, which in some patients ascends to the kidneys and enters the bloodstream (urosepsis). Antibodies in sera usually act to defend against infections, but studies into other Gram-negative bacteria have identified patients expressing antibodies capable of inhibiting complement-mediated killing (Wells et al, 2014). These inhibitory antibodies were characterised as IgG2 specific to the O-antigen component of lipopolysaccharide (LPS) coating the bacteria surface. It was hypothesised that patients suffering with urosepsis may express O-antigen specific IgG2 antibodies capable of inhibiting the serum-mediated killing of *E. coli*, promoting progression of pathology.

Methods

45 patients with *E. coli* infection and urosepsis were screened for the presence of IgG2 specific for their cognate strain's LPS. LPS extractions were confirmed by silver staining. Specific antibody titres were measured by ELISA. The complement-mediated inhibition was confirmed by serum bactericidal assays.

Results

Over half the patients had detectable titres of IgG2 specific for the O-antigen region of LPS. Eleven patients had titres high enough to inhibit complement-mediated killing of *E. coli* isolates by Healthy Control Serum (HCS). These eleven isolates were statistically more sensitive to HCS than isolates cultured from patients without inhibitory antibody.

Conclusion

This suggests the presence of inhibitory antibody may be an important factor in progression of urinary tract infections to sepsis and could provide the basis for improved patient outcome via detection of inhibitory antibodies and subsequent treatment development.

1. Wells TJ, Whitters D, Sevastsyanovich YR, et al. Increased severity of respiratory infections associated with elevated anti-LPS IgG2 which inhibits serum bactericidal killing. J Exp Med 2014; 211(9): 1893-904.

34

Overcoming antimicrobial resistance – exploiting zinc intoxication to restore antibiotic efficacy

Erin B. Brazel[1], **Stephanie L. Begg**[1], **Bart A. Eijkelkamp**[1], **Ibrahim M. El-Deeb**[2], **Trent Conroy**[2], **James C. Paton**[1], **Alastair G. McEwan**[3], **Jason W. Rosch**[4], **Mark von Itzstein**[2], **Mark J. Walker**[3], **Christopher A. McDevitt**[1]

1. Research Centre for Infectious Diseases, University of Adelaide, Adelaide, SA, Australia

2. Institute for Glycomics, Griffith University, Southport, QLD, Australia

3. School of Chemistry and Molecular Biosciences and Australian Infectious Diseases Research Centre, The University of Queensland, St Lucia, QLD, Australia

4. Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, United States

The prevalence of antibiotic resistant pathogens continues to rise and threatens to disrupt healthcare on a global scale. To combat antibiotic resistance, novel strategies for treating bacterial infections are urgently required. The metal ion zinc has a critical role in innate immune defence and its deficiency is associated with a marked increase in susceptibility to bacterial infections. Although the molecular basis for the antimicrobial activity of zinc remains to be elucidated, recent studies have shown that phagocytic cells exploit zinc intoxication as a component of their bacterial clearance mechanisms. *Streptococcus pneumoniae* (the pneumococcus) is a major cause of local and invasive diseases and is associated with significant human mortality. Despite the importance of zinc at the host-pathogen interface, the impact of zinc stress on *S. pneumoniae* remains poorly understood. Here, we investigated how zinc stress affected the virulent *S. pneumoniae* D39 strain using a combination of phenotypic growth, cellular metal accumulation and macrophage survival analyses. These studies revealed that *S. pneumoniae* encoded a cation diffusion facilitator family transporter (CzcD) that was capable of zinc efflux and contributed to pneumococcal survival within phagocytic cells. We further examined the impact of zinc stress by abolishing *czcD* functionality. This revealed that zinc intoxication rendered *S. pneumoniae* more sensitive to specific classes of antibiotics. Building on these findings, we examined synergism between zinc and antibiotics using ionophores to increase the potency of zinc stress. Ionophore-mediated zinc treatment restored antibiotic susceptibility to the multidrug resistant *S. pneumoniae* 23F strain. Collectively, this study provides detailed insight into zinc resistance in *S. pneumoniae* and highlights the therapeutic potential of zinc and ionophores as adjuvants to antibiotics as a novel treatment strategy.

35

Pan-genome of the livestock gut-associated *Streptococcus bovis/Streptococcus equinus* complex

Rosalind A Gilbert[1, 2], **William J Kelly**[3], **Diane Ouwerkerk**[1, 2], **Athol V Klieve**[2, 4]

1. Department of Agriculture and Fisheries, Queensland Government, Brisbane, Qld, Australia

2. QAAFI, The University of Queensland, Brisbane, Qld, Australia

3. Donvis Ltd, Palmerston North, New Zealand

4. School of Agriculture and Food Science, The University of Queensland, Gatton, Qld, Australia

The *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC) is a group of animal and human-derived commensal bacteria, found in the rumen and gastrointestinal tract. These fast-growing organisms can over-proliferate in the rumen of cattle when they transition too quickly from forage to high grain diets, such as those employed in the feedlot industry. SBSEC strains are also opportunistic pathogens impacting on the health of ruminant livestock, having been associated with mastitis in dairy cattle and laminitis in cattle and horses. A pan-genome, incorporating the complete bacterial genome sequences of 39 predominantly livestock-derived SBSEC isolates (representing *S. bovis*, *S. equinus*, *S. henryi*, and *S. gallolyticus*) was developed. This pan-genome enabled the mechanisms which may contribute to the over-proliferation of these organisms within the rumen to be identified, including (a) compounds for inhibiting the growth of other microbes (bacteriocins), and (b) a wide variety of carbohydrate-degrading enzymes which enable these organisms to rapidly utilize high grain diets. The majority of SBSEC examined possessed genes coding for enzymes classified within eight glycosyl hydrolase families, capable of breaking down complex plant carbohydrates. The bacteriocin genes identified were primarily bacteriocin class II lantibiotics, however strain-specific bacteriocins were also noted. The identification of restriction-modification and CRISPR/cas systems also provided insights into how these organisms may defend themselves against phage infection. Despite the presence of these defense systems, 22 prophage-associated sequences, encoding a sufficient complement of phage genes to be designated as "intact" prophages, were identified within the pan-genome. Further examination of 13 *S. equinus* strains using proteomics and TEM, showed that at least three of these prophages produced intact phage particles. The establishment of an open, SBSEC pan-genome has therefore provided novel insights the extent of diversity, or lack thereof, found within gut-associated SBSEC isolates sourced from livestock around the globe.

Oral Abstracts

36

The rumen microbiome and its functional relationship with the host animal

Stuart Denman[1]

1. CSIRO, Brisbane, QLD, Australia

The complex relationship between the host ruminant and its inhabitant microbiota has been the focus of research for decades initially centred around identification of the types of microbiota that reside within the rumen through to a greater understanding of their functional contribution to the host's energy requirements. As new technology has become available, what originally involved the isolation and detailed studies of single strains in the laboratory has now moved to large scale sequencing of "total" rumen microbiota nucleic acids (genomics and transcriptomics), proteomics and metabolomics. Notwithstanding the limitations of these new techniques, the adoption of these techniques has been rapid and applied to most ruminant systems. Initially to define the variance in rumen bacterial populations on diet shifts from forage based diets to those with higher proportions of grain. Most notable in the last decade the emphasis has been focused around the understanding of the rumen microbiota's contribution to agricultural greenhouse gas emissions, predominantly methane. While now there is increased interest on defining the rumen microbiota of an efficient production animal (meat and dairy) and the influence of the host genetics on shaping the microbiota of the rumen.

37

Koala retrovirus infection in Queensland and South Australian koala populations

Joanne Meers[1], Nishat Sarker[1], Richard Emes[2], Jessica Fabijan[3], Farhid Hemmatzadeh[3], Jasmeet Kaler[2], Helen Owen[1], Jennifer Seddon[1], Greg Simmons[1], Natasha Speight[3], Rachael Tarlinton[2], Darren Trott[3], Lucy Woolford[3]

1. School of Veterinary Science, University of Queensland, Gatton, QLD, Australia

2. School of Veterinary Medicine and Science, The University of Nottingham, Leicestershire, England

3. School of Animal and Veterinary Science, The University of Adelaide, Roseworthy, South Australia, Australia

Koala populations are in decline in many regions of Australia, with disease, injury and habitat loss the main causes. Koala retrovirus (KoRV) is considered an important threat to koala health and survival. The nature of KoRV infection is complex, with apparently different patterns of infection and disease in different regions of the country. We investigated the nature of KoRV infection in two distinct koala populations, sampling 105 koalas in South Australia (SA) and 71 koalas in Queensland (QLD). The study analysed proviral (DNA) and plasma viral (RNA) levels, the completeness of proviral inserts and expression of viral genes, and the diversity of nucleotide sequence of the *env* gene. The association of these parameters with disease syndromes was investigated. SA koalas had lower mean proviral and viral loads than QLD koalas and many SA animals apparently possessed incomplete proviral inserts and expressed only some viral genes. There was an association between viral RNA loads and the presence of neoplasia in both populations, and QLD koalas with clinical chlamydiosis had higher proviral and viral loads than healthy QLD koalas. Diversity of env subtypes was high within individual koalas in both populations, in both proviral DNA in cells and in expressed viral RNA in plasma. KoRV-A was the predominant proviral subtype in SA and QLD koalas, and although it was also the predominant RNA subtype in SA, subtypes B and D were the predominant RNA subtypes in QLD. In contrast to findings of others, KoRV-B was not associated with neoplasia in either population. However, other subtypes in either proviral DNA or viral RNA forms showed associations with chlamydiosis and neoplasia. These results demonstrate even greater complexity of the host-virus relationship between KoRV and koalas than previously recognized. Hypotheses on the endogenous versus exogenous nature of KoRV infection in different koala populations are supported by some, but not all of our findings. Better understanding of this virus has potential to aid koala conservation programs.

38

Effect on the rumen microbiomes of cattle shifting from grazing extensive pastures to floodplain pastures in the Northern Territory.

Diane Ouwerkerk[1, 2], Rosalind Gilbert[1, 2], Windu Negara[3], Tim Schatz[4], Athol Klieve[3, 2], Kieren McCosker[5]

1. Department of Primary Industries and Fisheries, Dutton Park, QUEENSLAND, Australia

2. Queensland Alliance of Agriculture and Food Sciences, University of Queensland, St Lucia, Queensland, Australia

3. School of Agriculture and Food Sciences, University of Queensland, Gatton, QUEENSLAND, Australia

4. Department of Primary Industries and Resources, Darwin, Northern Territory, Australia

5. Department of Primary Industry and Resources, Katherine, Northern Territory, Australia

The majority of beef cattle production in northern Australia is extensively grazed on unimproved pastures. In the Northern Territory cows which are surplus to breeding requirements (culled cows) are sold and the revenue generated can have a large influence on the profitability of the beef enterprise. Shifting healthy but underweight culled cows to graze on floodplain pastures to improve their growth performance and carcase characteristics has been identified as having the potential to increase the profitability of culled cows. The effect of shifting cows grazing native pastures to grazing floodplains on the rumen microbiome was investigated in 41 cows sourced from either commercial (COM) properties (32 cows) or Beatrice Hill Research Station (BHRS) (9 cows). Rumen fluid samples obtained from animals at induction to the floodplain (Day 0), and again at Days 34 and 137 were used for microbial diversity profiling (barcoded V3-V4 16S rRNA gene amplicon). Sequencing was done using the Illumina MiSeq platform and the sequence data was analysed using the QIIME 1.9 software package. Four alpha diversity measures (Chao1, Shannon, Observed species and Phylogenetic diversity whole tree) were significantly different between COM and BHRS cows at Day 0 with reduced diversity in COM cows. However by Day 34 the measures showed the number of observed microbial species for the COM cows had reached similar levels to the BHRF cows. Following a further 103 days on the floodplain, the extent of microbial diversity remained similar across the herd. The taxonomic composition of the rumen microbiomes was significantly different by Day 137 to the composition at Day 0 indicating that diet is one of the primary drivers in determining the relative taxonomic composition of the rumen, even in a relatively uncontrolled, extensively grazed feeding system.

Oral Abstracts

39

Cyanobacteria in dryland rehabilitation

Angela M Chilton[1, 2], Miriam Muñoz-Rojas[4, 5, 3], Brett A Neilan[6, 2]

1. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia

2. Australian Centre for Astrobiology, University of New South Wales, Sydney, NSW, Australia

3. Centre for Ecosystem Science, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW, Australia

4. Kings Park and Botanic Garden, Kings Park, WA, Australia

5. School of Biological Sciences, University of Western Australia, Crawley, WA, Australia

6. School of Environmental and Life Sciences, University of Newcastle, Callaghan, NSW, Australia

Drylands encompass 40% of Earth's terrestrial surface and are set to increase under predicted climate models. In addition to being biodiversity hot-spots, they sustain large human populations and a range of key industries including agriculture, mining, and tourism. Inevitably, there is often a clash between maintaining ecological productivity and land degradation through continued economic use. Current rehabilitation strategies include the regulation of livestock, artificial enrichment of soils, and reinstatement of native vegetation. However, overlooked in these processes are the critical roles performed by microorganisms. Cyanobacteria are a globally distributed phylum of photosynthetic bacteria which, in drylands, form integral components of biocrusts - topsoil assemblages of microorganisms, mosses and lichens that stabilise the surface and enrich the soil profile. Able to tolerate extreme arid conditions while performing critical ecosystem services, biocrust cyanobacteria offer promising solutions to several hurdles currently limiting dryland rehabilitation. We seek to harness this potential in a multifaceted approach to improve restoration success rates.

Using next-generation sequencing, we have profiled biocrust microbiomes from across Australia and identified key cyanobacterial species involved in their formation and maintenance. Our datasets illustrate the natural status of biocrusts and help establish informed targets to assess and monitor topsoil recovery. Seasonality of precipitation was identified as a key factor affecting biocrust assembly on an intra-continental scale, indicating biocrust restoration will rely on employing locally-adapted, endemic cyanobacterial strains. In addition, we have isolated key biocrust species and have conducted novel microcosm experiments examining the effect of cyanobacteria on seedling establishment. We performed bio-priming of seeds with indigenous cyanobacteria and showed this had significant positive effects on the germination and seedling growth of *Acacia hilliana* and *Senna notabilis*, two native species used in restoration. Our work highlights the importance of cyanobacteria in drylands and is developing practical approaches for their integration with current rehabilitation strategies to enhance ecological outcomes.

40

Population and evolutionary dynamics of Shiga-toxin Producing *Escherichia coli* O157 in an Australian beef herd

Meghan Jones[1], Sophie Octavia[1], Geraldine Lammers[2], Jane Heller[2], Ruiting Lan[1]

1. University Of New South Wales, Sydney, NSW, Australia

2. Charles Sturt University, Wagga Wagga, NSW, Australia

Shiga toxin producing *Escherichia coli* O157:H7 (STEC O157) is naturally found in the gastrointestinal tract of cattle and can cause severe disease in humans. There is limited understanding of the population dynamics and microevolution of STEC O157 at herd level. In this study, isolates from a closed beef herd of 23 cows were used to examine the population turnover in the herd. Of the nine STEC O157 clades previously described, clade 7 was found in 162 of the 169 isolates typed. Multiple locus variable number tandem repeat analysis (MLVA) analysis differentiated 169 isolates into 33 unique MLVA types. Five predominant MLVA types were evident with most of the remaining types containing only a single isolate. MLVA data suggest that over time clonal replacement occurred within the herd. Genome sequencing of 18 selected isolates found that the isolates were divided into four lineages, representing four different 'clones' in the herd. Genome data confirmed clonal replacement over time and provided evidence of cross transmission of strains between cows. The findings enhanced our understanding of the population dynamics of STEC O157 in its natural host that will help developing effective control measures to prevent the spread of the pathogen to the human population.

41

Not available at time of printing

Maria Liaskos[1]

1. La Trobe University, Bundoora, VIC, Australia

Not available at time of printing

42

Tuberculosis: New therapies for an old enemy

Jamie Triccas[1], Claudio Counoupas[1], Diana Quan[1], Warwick Britton[2], Peter Rutledge[3]

1. Infectious Diseases and Immunology, University of Sydney, Sydney, NSW, Australia

2. Mycobacterial Research Program, Centenary Institute, Sydney, NSW, Australia

3. School Of Chemistry, University of Sydney, Sydney, NSW, Australia

Tuberculosis (TB) remains a major cause of mortality and morbidity worldwide, and more effective strategies are required to control infection. The current vaccine, BCG, is only partially effective against TB, and drug-resistant strains of *Mycobacterium tuberculosis* are emerging at an alarming rate. The past decade has seen an explosion in the development of new TB vaccine and drug candidates, with a number now in clinical trials. The entry of new candidates into this TB vaccine 'pipeline' requires the rational design of novel vaccines effective against multiple stages of *M. tuberculosis* infection (i.e. target latent TB). We have used genetic screens to identify novel antigenic targets of *M. tuberculosis* for incorporation into new vaccines. These studies have identified candidate antigens that protect against pulmonary *M. tuberculosis* infection in pre- and post-exposure animal models, and are strongly recognised by the immune system of TB patients. We have also undertaken a program of drug discovery by screening natural product libraries for antimycobacterial activity. We identified samples from marine organisms with exceptionally potent activity against drug-susceptible and drug-resistant strains of *M. tuberculosis*. These samples were non-toxic against human cell lines, could work in synergy with existing TB drugs, and were able to inhibit intracellular growth of *M. tuberculosis*. Our current focus is to prepare our most promising vaccines and drugs for assessment of efficacy against TB in human trials.

43

Neisseria gonorrhoeae vaccine development – targeting of minor outer membrane proteins MetQ and NHBA to elicit host immunity

Evgeny Semchenko[1], Kate L Seib[1]

1. Griffith Univeristy, Institute for Glycomics, Gold Coast, QLD, Australia

Neisseria gonorrhoeae (Gc) is an obligate human pathogen and the causative agent of the sexually transmitted infection gonorrhoea. There are over 106 million reported cases of gonorrhoea each year worldwide, however the actual number of infections is difficult to estimate due to high incidence of asymptomatic infections (up to 40% in men and 50-80% in women). Undiagnosed and/or untreated gonococcal infections can progress to severe sequelae, such as pelvic inflammatory disease (PID) in women, which can lead to infertility. In addition, infections with Gc also facilitate transmission of HIV. The Wold Health Organisation and Centres for Decease Control have both declared *N. gonorrhoeae* as an urgent threat to global health, due to increasing incidence and widespread antibiotic resistance. Development of a gonococcal vaccine has been challenging, as the bacterium is known for its high rate of antigenic variation as well as its ability to suppress protective immunity (i.e., natural infection with Gc offers no protection against subsequent re-infection). Therefore, vaccine development requires identification of suitable candidates that can induce a non-native, protective immune response.

We have characterised several minor components of the gonococcal outer membrane and investigated their potential use as vaccine antigens. Here we discuss two antigens, MetQ (methionine receptor) and NHBA (Neisseria heparin binding antigen), that were identified as potential vaccine targets using a reverse vaccinology approach. In *N. gonorrhoeae,* both of these proteins are surface exposed, immunogenic, highly conserved with minimal amino acid variation between strains, and stably expressed with no evidence of phase variation. Mutant strains lacking MetQ or NBHA have decreased adherence and invasion of cervical and urethral epithelial cells, as well as reduced survival in human serum, highlighting the importance of these surface molecules for gonococcal pathogenesis. Both anti-MetQ and anti-NHBA antibodies from mice elicit complement dependent bactericidal activity, and anti-NHBA antibodies also facilitate opsonophagocytic killing of Gc. NHBA from the closely related bacteria *Neisseria meningitidis* is present in the licensed multicomponent meningococcal serogroup B vaccine Bexsero, and we have determined that Bexsero induces antibodies in humans that recognise the gonococcal NHBA homologue. Work is ongoing to identify the full set of gonococcal targets recognized by Bexsero-induced antibodies, and their functional activity against gonorrhoea.

44

Reviewing the effects of chlamydial immunizations on the host immune system

David Lizarraga[1, 2], Scott Carver[1], Peter Timms[2]

1. School of Natural Sciences, University of Tasmania, Sandy Bay, Tasmania, Australia

2. School of Science and Engineering, University of Sunshine Coast, Sippy Downs, Queensland, Australia

Multiple host species can be targets of infection from bacterial species belonging to the *Chlamydiaceae* family, leading to devastating diseases such as conjunctivitis, pneumonia, and pelvic inflammatory disease. Antibiotics can alleviate symptoms of chlamydial infection, although many infections are asymptomatic making early detection difficult. Due to this, controlling the spread of chlamydial infection with antibiotics is a financially expensive undertaking that may not be practical for certain populations (e.g. underdeveloped countries or infected wildlife). Thus, developing a functional chlamydial vaccine has been a major focus of chlamydial research over the last six decades. Despite many reviews describing published chlamydial immunizations and their effects on host immune parameters (ex. immune cell, cytokine, and antibody abundance), there exists a need for a systematic comparison of these immunizations. Using the database Web of Science, we searched the literature for studies containing chlamydial immunizations and measurements of host parameters. The resulting 389 studies were further filtered based on the content of the abstract to exclude non-related studies. Within each study, immune parameter measurements from immunized and control groups were extracted and used to calculate an effect size. We applied statistical methods to compare effect sizes between and among studies. There exists an increase in the number of published chlamydial immunization studies, particularly in the last 20 years. Not surprisingly, our study identifies mice as the host most commonly used for chlamydial immunization experiments. After averaging the effect sizes of immune parameters, we found statistically trending decreased chlamydial loads and increased immunoglobulin G (IgG) antibodies after immunization. Preliminary results suggest the average abundance of interferon gamma, IgA, IgG1, and IgG2a were equivocal after immunization. Additionally, some immune parameters have an asymmetrical distribution of effect sizes suggestive of publication bias. The results of our meta-analysis identify trends in chlamydial immunization research that will guide future studies aimed at developing a functional chlamydial vaccine.

Oral Abstracts

45

Identification of small molecules as novel therapeutic options that suppress virulence in *Neisseria gonorrhoeae* and increase susceptibility to CAMPs

Christopher A Mullally[1], Keith Stubbs[1], Anandhi Anandan[1], Genevieve Evans[1], Martin Scanlon[2], Gary A Jarvis[3], Constance John[3], Kathy Fuller[1], Alice Vrielink[1], Charlene M Kahler[1]

1. University of Western Australia, Perth, W.A., Australia

2. Monash University, Melbourne, Victoria, Australia

3. University of California San Francisco, San Francisco, California, United States of America

Neisseria gonorrhoeae is an exclusively human pathogen that most commonly infects the urogenital tract resulting in gonorrhoea. Empirical treatment of gonorrhea requires antibiotics, but multi-drug resistance has occurred to all first-line treatments thus resulting in the imperative to find new treatment options. The enzyme lipooligosaccharide phosphoethanolamine transferase A (EptA) is responsible for the addition of phosphoethanolamine (PEA) to lipid A as it is transported through the periplasm to the outer membrane. The addition of PEA to lipid A is essential for bacterial resistance to cationic antimicrobial peptides (CAMPs) and for attachment to human epithelial cells. We hypothesised that small molecules that inhibit EptA will result in increased sensitivity to CAMPs and enhance natural clearance of gonococci via the human innate immune response.

A library of 250 compounds has been synthesised and tested using an *in vitro* microbroth dilution assay against the reference strain *N. gonorrhoeae* FA1090. Seventy-four of these compounds have enhanced the sensitivity of strain FA1090 to polymyxin B (PxB), a CAMP. Compound 2B7 increased the sensitivity of strain FA1090 to PxB by 4-fold. MALDI-TOF MS analysis of lipid A extracted from FA1090 cells treated with the compound revealed a ~20% decrease in PEA decoration of the lipid A when compared to untreated bacteria. This was validated by a cytokine assay using THP-1 cells exposed to wild-type LOS and LOS from bacteria treated with the compound. The LOS from the compound treated cells showed a 55% decrease in TNFα induction consistent with reduced cytotoxicity resulting from the inhibition of PEA decoration of lipid A. To further confirm these observations a fluorescent assay using dansyl-polymyxin is being trailed.

In conclusion, small molecules can be designed to bind and inhibit EptA and can sensitise *N. gonorrhoeae* to killing by cationic antimicrobial peptides.

46

Improving tetanus toxoid vaccine production

Nicolas Zaragoza[1], Camila A. Orellana[1], Cuauhtemoc Licona-Cassani[1], Glenn Moonen[2], George Moutafis[2], John Power[2], Esteban Marcellin[1]

1. AIBN, Brisbane, QUEENSLAND, Australia

2. Zoetis, Melbourne, Victoria, Australia

Toxoid vaccines are used routinely in the livestock industry to prevent animal-disease caused by pathogenic clostridia. *Clostridium* toxoid vaccines are produced using batch fermentation processes with complex mediums, and the produced toxin is then inactivated with formalin to form the toxoid. However, the lack of industrial process reproducibility due to media preparation and composition results in random titres and sporadic batch failures, causing great economic losses. Here, we applied a systems biology approach to study *Clostridium tetani* potent neurotoxin production, responsible for the life-threating tetanus disease. We show that time-course transcriptomics and metabolomics data allowed discovering key components in toxin production regulation. We initially found that the addition of these five metabolites to the medium inhibited toxin production. However, the optimization of each component concentration resulted in a two-fold increase in toxin production. Our results demonstrate that toxin expression is tightly regulated by these components, as small changes in the metabolites concentration drastically changed from toxin inhibition to high productivity. We anticipate that these findings will help the industry to achieve much higher titres than those obtained today and to restore poor batches performance.

47

Ticks in Australia: endemics; exotics; which ticks bite humans?

Stephen C Barker[1], Dayama Barker[1]

1. Discipline of Parasitology, School of Chemistry & Molecular Biosciences, University of Queensland, St Lucia, QLD, Australia

At least 71 species of ticks occur in Australia (Barker et al 2014 *IntJParasit*,12,941-; Ash et al 2017 *Parasit&Vectors10*,70-). A further 33 or so species are endemic to our neighbours New Guinea and New Zealand. The ticks of Australasia are phylogenetically distinct. Indeed, there are at least two lineages of ticks that are unique to Australasia: the sub-family *Bothriocrotinae*, Klompen, Murrell & Barker 2002; and the new genus *Archaeocroton* Barker & Burger 2018. *Ixodes holocyclus*, the eastern paralysis tick, is notorious for biting humans on the east coast of Australia. The toxins of this tick seem to be the most potent of all tick-toxins, indeed there have been at least 20 fatalities (Barker & Walker 2014 *Zootaxa*3816). To provide context to the number of fatalities caused by *I. holocyclus*, there have been comparable numbers of fatalities from red-back spiders (n=18) and funnel-web spiders (n=13, Sutherland & Tibballs 2001 OxfordUnivPress). Thankfully, deaths from the bite of *I. holocyclus* are now rare due to the advent of intensive care-units in regional hospitals and expert medical treatment. Not so, for dogs and cats, since the treatment of tick-paralysis in dogs and cats is difficult, and thus many be extremely expensive. But a new generation of tick-preventative for dogs, but not cats, provides unprecedented protection against *I. holocyclus*. The illnesses that *I. holocyclus* has been associated with include Australian multi-system disorder, post-infection fatigue, auto-immune disease, paralysis, allergies (particularly to the bites of larvae), Queensland Tick Typhus (*Rickettsia australis*), mammalian meat allergy and tick anaphylaxis. Professor

Graves, the speaker following me, will address some of these illnesses. In WA and on Yorke Peninsular, SA, *Amblyomma triguttatum triguttatum*, the ornate kangaroo, tick is the villain (*Rickettsia gravesii* with a nasty bite) whereas on Flinders Island and other parts of Southern Australia the troublesome tick is *Bothriocroton hydrosauri*, the southern reptile tick (*Rickettsia honei*, Flinders Island Spotted Fever). Lastly, *Ornithodoros capensis*, the sea bird soft tick, is notorious for the very large number of different viruses that have been isolated from this tick (Barker & Walker 2014, *Zootaxa*3816).

48

Tick-borne infectious diseases in Australia.

Stephen R Graves[1]

1. Australian Rickettsial Reference Laboratory, Geelong, VIC, Australia

Tick bites in Australia can lead to a variety of illnesses in patients. These include infections, allergies, paralysis, auto-immune disease, post-infection fatigue and Australian multi system disorder. This talk is limited to infections.

There are 3 important ticks that bite humans and can transmit infections:

1. *Ixodes holocyclus* (paralysis tick) and related species *I.tasmani* (common marsupial tick) and *I.cornuatus* (southern paralysis tick).
2. *Amblyomma triguttatum* (ornate kangaroo tick).
3. *Bothriocroton hydrosauri* (southern reptile tick).

There are 2 important groups of human pathogenic bacteria transmitted by ticks in Australia:

a. rickettsial infections. These include Queensland Tick Typhus (*Rickettsia australis*), Flinders Island Spotted Fever (*R.honei*) and Australian Spotted Fever (*R.honei* subs. *marmionii*). Murine typhus (*R.typhi*) and Cat flea typhus (*R.felis*), are rickettsial infections transmitted by fleas, not ticks and Scrub typhus (*Orientia tsutsugamushi*), transmitted by mites, not ticks, also occur in Australia.

b. Q Fever caused by *Coxiella burnetii*. While normally transmitted to human via aerosols from infected vertebrate animals (eg cattle, sheep, goats), some ticks also carry this bacterium and can infect humans when they bite.

There are likely to be other, unknown human pathogens yet to be discovered in Australian ticks eg viruses, protozoa and other bacteria.

49

The search for Australia's tick-borne disease causing pathogens

Peter Irwin[1]

1. Murdoch University, Murdoch, WA, Australia

The metagenomics revolution has started to unravel the mysteries of microbial communities (comprising viruses, bacteria and eukaryotic microbes) within northern hemisphere ticks where arthropod co-infections and co-transmission of pathogens to vertebrate hosts are well known. Increasingly the role of endosymbionts as modulators of that transmission, and as potential pathogens in their own right is being recognised. What is the situation in Australia where concern about tick-borne illness in humans gathers apace, and microbiome research offers the potential to inform this contentious debate? Despite sharing some taxa with Ixodidae of other continents, approximately 65 endemic tick species are confined to the Australian continent, its offshore islands and New Guinea, and have evolved with the unique mammalian and monotremal fauna for millennia. Studies of Australian indigenous ticks, collected nationwide from the environment and a wide range of vertebrate hosts (including humans), and tested by high-throughput next generation sequencing technology, are starting to reveal rich and diverse microbiomes. Endosymbionts (e.g. *Candidatus* Midichloria mitochondrii, *Coxiella*, *Francisella* and *Rickettsia* spp.) are prevalent in ticks. These and the genera of familiar pathogens (e.g. *Anaplasma*, *Babesia*, *Borrelia*, *Ehrlichia*, and *Neoehrlichia* spp.) have now been identified locally, yet are distinct from their northern hemisphere relatives. We stand on the threshold of gaining a deeper understanding of the microbes of Australian ticks, which might provide answers to important questions about their disease-causing potential.

50

Protozoan expelled food vacuoles are an unrecognized vector for the transmission of cholera

Diane McDougald[1, 2], **Gustavo Espinoza Vergara**[1, 3], **Parisa Noorian**[1], **Shuyang Sun**[1]

1. University Of Technology Sydney, SYDNEY, NSW, Australia

2. Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

3. School of Biotechnology and Bimolecular Science, University of New South Wales, Sydney, NSW, Australia

Bacterivorous protists (protozoa) frequently release undigested bacteria in egested food vacuoles (EFV), which have, until now, been an unrecognized vector for the transmission of cholera. Here we used confocal microscopy, exposure to stresses (pH and antibiotics) and the infant mouse model of colonisation to investigate stress resistance and infection potential of cells contained in the EFVs.

Our results show that *Tetrahymena pyriformis*releases large numbers of EFVs when feeding on *Vibrio cholerae*. EFVs were demonstrated to be extremely stable in artificial seawater with no significant loss of viability after long term storage at room temperature. Cells within the EFVs were not affected by incubation at low pH (3.5) or in the presence of antibiotics. When incubated at 37°C, the cells escaped very rapidly. Escaped cells were shown to have a fitness advantage over planktonic cells both *in vitro* and *in vivo*. These findings suggest that EFVs could facilitate the survival of *V. cholerae*in the environment under a variety of stressful conditions and that the EFVs would also protect *V. cholerae* as it transits through the stomach. In addition, the escaped cells have enhanced colonisation potential. The work also shows that cells within EFVs are primed to cause infection and may be a significant contributor to the dissemination of epidemic *V. cholerae*strains.

Oral Abstracts

51

Serological evidence for the non-human reservoirs of Ross River virus; Australia's most common arbovirus

Eloise Stephenson[1, 2], **Penny Rudd**[2, 3], **Lara Herrero**[2]

1. Griffith University, Main Beach, QLD, Australia

2. Institute for Glycomics, Gold Coast, QLD, Australia

3. Institute for Glycomics, Gold Coast, QLD, Australia

Introduction

Arboviruses account for 17% of the estimated global burden of infectious disease[1]. In Australia, the most common and widespread arbovirus in humans is Ross River virus (RRV), with ~4800 cases reported each year across all states and territories[2]. RRV is a significant public health and economic burden. In 2001, the estimated cost of RRV infections to the community was between $2.8 and $5.7 million annually[3]. RRV is maintained in enzootic cycles, and comparatively few studies have focused on identifying how species contribute to spread and amplification of the virus.

Methods

Here we present on findings from serological studies testing 400 wildlife serum samples using the gold standard, plaque reduction neutralization test[4]. Samples were collected through veterinary clinics over a 12 month period and species tested include common urban possums, horses, flying foxes and birds. Taking a multidisciplinary approach, we combine this data with ecological assessments of potential reservoirs.

Results

Our findings challenge the long-held dogma that marsupials are better reservoirs than placental mammals, which in turn are better reservoirs than birds. We discuss some of the advantages and disadvantages of using serological data to make assumptions on the non-human reservoirs of arboviruses.

Conclusion

These findings are significant for the further research and management of Ross River virus. This research highlights the importance of disentangling vector-host relationship for meaningful management strategies by drawing on multidisciplinary skills including microbiology, vector and animal ecology, public health and veterinary science.

1. Weaver SC, Barrett AD. Transmission cycles, host range, evolution and emergence of arboviral disease. Nat Rev Microbiol. 2004;2(10):789-801.
2. National Notifiable Diseases Surveillance System. Australian Government, Department of Health. 2017. http://www9.health.gov.au/cda/source/cda-index.cfm. Accessed 12 Sep 2017
3. Harley D, Sleigh A, Ritchie S. Ross River virus transmission, infection, and disease: a cross-disciplinary review. Clin Microbio Rev. 2001;14(4): 909-32.
4. Hobson-Peters J. Approaches for the development of rapid serological assays for surveillance and diagnosis of infections caused by zoonotic flaviviruses of the Japanese encephalitis virus serocomplex. BioMed Res Int. 2012;2012:379738.
5.

52

In vivo transcriptome of insect pathogen *Yersinia entomophaga* MH96.

Amber R Paulson[2, 1], **Xue-Xian Zhang**[3], **Paul B Rainey**[4, 5, 1], **Maureen O'Callaghan**[2], **Mark RH Hurst**[2]

1. New Zealand Institute for Advanced Study, Massey University at Albany, Auckland, New Zealand

2. Forage Science, AgResearch Limited, Lincoln, New Zealand

3. Institute of Natural and Mathematical Sciences, Massey University at Albany, Auckland, New Zealand

4. Microbial Population Biology, Max Planck Institute for Evolutionary biology, Plön, Germany

5. Laboratoire de Génétique de l'Evolution, ESPCI ParisTech, Paris, France

Pathogenic bacteria have evolved specialized virulence factors (VFs) that enable entry and persistence within host tissues. Here we investigated the *in vivo* transcriptome of a novel insect pathogenic bacterium, *Yersinia entomophaga* MH96, to identify key virulence factors involved during different stages of infection (early, middle and late) and at different temperatures (25 and 37 °C) within the hemocoel of larval insect host, *Galleria mellonella*. Originally, *Y. entomophaga* was isolated from the cadaver of *Costelytra giveni* (Coleoptera: Scarabaeidae) larva, which is an endemic and notorious pasture pest of New Zealand. Development of *Y. entomophaga* as a biopesticide has proven consistent pathogenesis by *per os* challenge against *C. giveni*, as well as a wide range of coleopteran, lepidopteran, and orthopteran species. Additionally, a median lethal dose of at least three bacterial cells is sufficient to kill larvae of the greater wax moth *G. mellonella* within 4 days of injection with similar levels of mortality observed at both 25°C and 37°C. Here we use a novel method to enrich for pathogen transcriptional signal from extracellular *Y. entomophaga* within the hemolymph of *G. mellonella*. Sequencing results provided sufficient alignment of transcripts to the reference genome (9 – 91 %) enabling capture of the dynamic range of pathogen gene expression within the host. Striking temperature-dependent regulation was observed for several key VFs, including the insecticidal toxin complex (Yen-TC), type VI secretion system and flagellum, all of which were completely down-regulated at 37 °C compared to 25 °C. These data are driving focused investigations into the temperature-dependent regulation of Yen-TC as well as the characterization of RNA-binding proteins that were shown to be up-regulated during early infection and at 37 °C in the host. This work provides critical insight into the pathobiology and *in vivo* gene expression of a potentially important biopesticide.

Oral Abstracts

53

Rapid and smart diagnostics for low resource detection of human and animal diseases

Joanne Macdonald[2, 1]

1. Genecology Research Centre, Inflammation and Healing Research Cluster, School of Science and Engineering, University of the Sunshine Coast, Sippy Downs, Qld, Australia

2. Division of Experimental Therapeutics, Columbia University, New York, NY, USA

Existing and emerging diseases threaten human health, food security, livestock and agricultural productivity. However, point of care methods for rapid detection of these diseases can be limited by accuracy, requiring centralised laboratory testing using accurate molecular genetics-based diagnostics, such as real-time PCR. The requirement for centralised testing can create critical delays in the treatment and management of infectious diseases outbreaks. I have identified and developed a low-resource molecular genetics testing workflow that enables accurate disease identification without any sophisticated equipment. Not even a centrifuge is required to process specimens, only a battery-operated heating block is required for incubation at 39 °C during an isothermal amplification step. Pathogens can be detected within raw samples (blood, tissue or swabs) in 20-40 minutes with similar sensitivity to laboratory-based molecular genetics tests (10-100 copies/µL). My research group has demonstrated this method can be applied generically for the detection of bacteria, parasites, and viruses, with high (>95%) diagnostic sensitivity, specificity, and accuracy. We have also developed multiplex technology to display disease identification in text without the requirement for electronic displays to interpret the results. Critically, only a single solution change enables adaption of the kits to detect other diseases, without any other change in the manufacturing protocol. The technology thus has broad health implications for improving accessibility of diagnostics in regional and remote communities, and for rapid deployment during emergent disease outbreaks.

54

Novel insights on the genomic RNA packaging and assembly of HIV

Johnson Mak[1]

1. Burnet Institute, Melbourne, VIC, Australia

Formation of HIV particles is driven by $Pr55^{Gag}$ precursor protein, and $Pr55^{Gag}$ is also the critical determinant to select HIV RNA genome for virion packaging. Full-length recombinant HIV $Pr55^{Gag}$ proteins have not been available in the past 30 years due to technical limitation, making it difficult to precisely define the biophysical and biochemical properties that drive the formation of HIV particles. We have since generated large amount of full-length recombinant HIV $Pr55^{Gag}$ protein for biochemical and biophysical analyses. This presentation comprises of a number of publications from our recent past, detailing how we have defined the protein and RNA requirement on genomic RNA packaging, plus the interplay amongst Gag oligomerization, lipids, RNA, and the thermodynamic properties that drive the formation HIV particles. These discoveries have revealed both novel and practical tools that can be used for the development of antiviral against HIV.

55

Human papillomavirus and cervical cancer control in Australia

Dorothy A Machalek[1]

1. The Royal Women's Hospital, Melbourne, VICTORIA, Australia

The development of efficacious human papillomavirus (HPV) vaccines that provide high levels of protection against infection with HPV – the cause of most cases of cervical cancer worldwide – is arguably one of the biggest population health innovations of the 21st century. Australia was the first country to introduce a publicly-funded national vaccination program in 2007, delivering three doses of the quadrivalent HPV vaccine to schoolgirls aged 12–13 years, with a 'catch-up' (2007–2009) for females up to 26 years. Schoolboys were added to the program in 2013.

Since implementation, Australian surveillance data has provided growing evidence for the population-level benefits of HPV vaccination, both direct, and through herd protection of those who remain unvaccinated. These benefits include rapid and substantial reductions in the prevalence of vaccine targeted HPV genotypes, diagnoses of genital warts and incidence of high-grade screen detected abnormalities, evident soon after program implementation. More recent surveillance data have found that vaccine-targeted HPV genotypes were nearing elimination among young women.

Prompted by the impact of vaccination and emerging evidence on cervical HPV testing, on December 1st, 2017, Australia transitioned to 5-yearly primary HPV screening, which is expected to further reduce cervical cancer incidence and mortality rates. Now is a time of great excitement in HPV control, with better vaccines (targeting more HPV types) and improved screening strategies (shifting from cytology to detection of HPV-DNA). Australia has been viewed as a global leader in both introducing new HPV prevention measures, and successfully demonstrating their impact. The research demonstrates that there is potential to eliminate one of the world's most common viruses, and a major cause of cancer in women.

56

HIV-1 Env trimers eliciting antibody with neutralising and cellular-dependent functions in vaccinated cows

Natalia Salazar-Quiroz[1], Jack Edwards[1], Behnaz Heydarchi[1], Brian Muller[2], Christopher Gonelli[1], Charlene Mackenzie[1], Georges Khoury[1], Damian F Purcell[1]

1. Department of Microbiology and Immunology, University of Melbourne, Parkville, VIC, Australia

2. Reef Pharmaceuticals, Melbourne, VIC, Australia

Oral Abstracts

Introduction: An effective HIV-1 vaccine that prevents transmission likely requires the stimulation of envelope (Env) antibodies capable of binding and neutralising a broad range of cell-free infectious virus particles, and to also stimulate antibodies that direct cellular-effector functions, such as antibody dependent cellular cytotoxicity (ADCC) and antibody dependent phagocytosis (ADP), to eliminate HIV cell-cell transmission. Authentic structured Env trimers have superior structural properties to elicit these antibodies; it is unclear, however, if more flexible uncleaved Env gp140 trimers are superior to cleaved SOSIP gp140 trimers for triggering, in one hand, neutralising activity by binding to epitopes such as the CD4 binding site (CD4bs) found on cell-free virus, and in other hand, antibodies targeting the CD4-induced epitopes present on cell-associated Env and utilized in ADCC or ADP. Vaccination of bovines with HIV-1 Env trimer vaccines have demonstrated unusual capability to produce these useful antibodies (Kramski et al., 2012; Sok et al., 2017). Here we compared HIV Env gp140 vaccines with different primary amino acid sequence, different glycosylation levels and different structural rigidity for their capacity to elicit antibodies with broad cross-clade binding and neutralising activity, especially targeting the CD4bs, and antibodies directing cell-associated immune functions.

Methods: A group of 32 cows were vaccinated with HIV-1 Envs from clades A, B and C before conception, and boosted during pregnancy in a period of 59 weeks. The Env binding activity was determined by direct and by antigen capture ELISA. The neutralising activity of bovine antibodies was assessed against a panel of pseudoviruses in TZM-bl or $CF2^3$ cells. Fc gamma receptor dimer ELISA was performed to investigate the interaction of bovine antibodies with human gamma receptors in immune cells. ADP-SHIP assay was also performed on high Fc receptor binder bovine antibodies.

Results: Analysis of sera pre- and post-vaccination and colostrum showed high titres of HIV Env-specific antibodies induced by the vaccination. These antibodies are IgG and demonstrate high binding affinity for their autologous envelope vaccine, and especially, cows that received clade B uncleaved Env gp140 exhibit binding breadth. These IgG targeted the CD4bs, V2 and V3 loops within Env. Bovine antibodies elicited by the clade A SOSIP Env gp140 vaccine yielded the highest neutralising activity against heterologous viruses. Neutralising activity against B-clade Env was also elicited by the more highly glycosylated uncleaved AD8 Env gp140, compared to a low glycosylated Env (PSC89), but this vaccine also induced IgG with the highest binding to FcgRIIa, mediating phagocytosis of beads coated with Env gp140 on the surface by THP1 cells.

Discussion: The analysis of different HIV-1 Env protein vaccines showed that, although the antibodies elicited with clade A SOSIP cleaved Env gp140 had lowest binding to HIV Env proteins, they could neutralise HIV pseudoviruses strongly. On the other hand, high binding of antibodies from cows vaccinated with the clade B, conformationally flexible, uncleaved Env gp140 trimers, did not guarantee efficient HIV-1 neutralisation, but these exhibited strongest binding to human FcgRIIa receptors on monocytes explaining the ADP activity in the THP1 model.

Conclusions: An optimal vaccine for stimulating antibody immunity to HIV may require both flexibility to present the Env epitopes triggered by interaction with CD4 on the surface of infected cells, and an elaborated structured neutralising epitope present on cell-free virion.

57

A multiple checkpoint HIV-1 inhibitor can block virus replication in vivo

David Harrich, Hongping Jin, Mary Lor, Lu Wang, dongsheng Li, li li, Zhi Ping Xu

Nullbasic (NB) is a mutant protein of the HIV-1 transcriptional activator protein, Tat. Our research has demonstrated that NB is a nontoxic, first-in-class antiviral agent that inhibits HIV production and viral spread in human T cells by independent mechanisms: 1) it inhibits the transcriptional activation function of Tat, 2) it disrupts HIV mRNA trafficking by interfering with the viral Rev regulatory protein, 3) it inhibits HIV reverse transcription. We have shown that with stable expression in cells, NB inhibits HIV replication in human cells and it also inhibits HIV reactivation from latently infected cells [1-6]. We used retroviral gene therapy vectors to deliver a Nullbasic-ZsGreen1 (NB-ZSG) fusion protein or ZsGreen1 (ZSG) to human CD4+ T cells, which were purified and transplanted into NOD-SCID or BALB/c-Rag2-/-γc-/- (RAG2) mice. The mice were infected with HIV-1 and virus replication was followed for up to 8 week. Both mouse models showed that Nullbasic inhibited virus replication. In Rag2 mice, NB-ZSG delayed replication and lowered viral titres by ~10-15 fold. Increased virus replication inversely correlated with NB-ZSG1expression in CD4+ T cells. Interestingly, NOD-SCID mice had CD4+ T cells that showed robust expression of NB-ZSG1 and up to 7,000-fold inhibition of HIV-1. As an adjunct method, we have trialled layered double hydroxide nanoparticles (LDH NPs) to deliver NB protein to primary human CD4+ T cells. We observed that 100% of CD4+ T cells can be treated with NB-LDH NPs. NB was detected in treated cells for three days. When HIV-1 infected CD4+ T cells were treated with NB-LDH NPs, a 90-fold decrease in HIV-1 mRNA was observed. Recent reports that the compound didehydro-cortistatin A can durably inhibit HIV-1 transcription by a mechanism conserved by NB suggests that long term inhibition of HIV-1 using NB-LDH NPs may be possible.

58

The Role of Exosomes During Infection with Bovine Herpesvirus 1

Tristan T Wimpenny[1], Tim T Mahony[1], Elizabeth E Fowloer[2]

1. QAAFI, Woolloongaba, QLD, Australia

2. DAF, Department of Agriculture and Fisheries, Woolloongaba, QLD, Australia

Bovine herpesvirus 1 (BoHV-1) is a ubiquitous pathogen of cattle and is implicated in many production limiting diseases. This virus is responsible for flu like infections in cattle worldwide. This infection can then, in turn, lead to secondary bacterial infections which are responsible for the development of severe disease complexes such as bovine respiratory disease (BRD). The virus has been divided into three genotypes; BoHV-1.1, BoHV-1.2a and BoHV-1.2b. Between and within these genotypes there is considerable variation in the capacity of BoHV-1 strains to cause disease, referred to as virulence. Currently, the molecular basis of the variation in virulence capacity between BoHV-1 strains is poorly understood. Additionally, it has been reported that the current Australian vaccine against BoHV-1 infection (Rhinogard™) has shown signs of loss of efficacy. Due to these factors, it is important to understand the interactions of the virus and cells during infection including miRNA production and, exosome interactions.

Exosomes are extra-cellular organelles believed to be involved with cell-to-cell communication and establishing anti-viral responses between cells. This anti-viral response is believed to be due to the transmission of viral miRNA between cells which, in turn, leads to the development of specific RNAi molecules.

Exosomes were isolated from bovine kidney cells after infection with Australia's oldest BoHV-1 isolate; V155. Isolation was performed via ultra-high-speed centrifugation methodology. The virus and exosome were pelleted and separated using a self-forming 60% optiprep gradient. The location of virus was confirmed via: reinfection, detection of BoHV-1 miRNA (miR-6), detection of glycoprotein C DNA and, detection of glycoproteins B and C. The location of exosomes was found via the detection of TSG101 and bovine CD63 proteins. Initial experimentation suggests the presence of viral miRNA or DNA encapsulated in the exosomes. To confirm this, cells were treated with exosomes before infection with a clonal virus of V155 (pBHVGFP) containing a GFP cassette. Infection was observed to determine the impact this treatment had on infection over time.

59

Variation in the capsular polysaccharide locus of *Streptococcus pneumoniae* isolates from low and middle-income countries in the Asia-Pacific

Sam Manna[1]

1. Murdoch Childrens Research Institute, Parkville, VIC, Australia

Streptococcus pneumoniae (the pneumococcus) is a leading cause of morbidity and mortality worldwide, causing otitis media, pneumonia, sepsis and meningitis in children, the elderly and the immunocompromised. Over 90 immunologically-distinct serotypes of pneumococcus have been reported, which vary in the biochemical structure of their capsule, encoded by the capsular polysaccharide (*cps*) locus. Accurate serotyping of pneumococci is required to track changes in serotype prevalence following vaccine introduction. Reference *cps* DNA sequences for the 90+ serotypes used for molecular serotyping are derived primarily from high-income countries. Data on serotype prevalence and distribution from high-income countries were a key driver in decisions surrounding the serotypes to be included in vaccines. These vaccines have subsequently been introduced into low and middle-income countries (LMICs), where limited data on pneumococcal population structure are often available. Our group has used DNA microarray to monitor serotype prevalence in LMICs in the Asia-Pacific region including in Fiji, Mongolia, Vietnam, Indonesia and Lao PDR. We identified two novel *cps* loci (designated 11F-like and 33F-like) in nasopharyngeal swabs both from healthy children and children with pneumonia. The genetic variation in the 11F-like *cps* locus results in changes to the antigenic properties of the capsule such that it does not encodes the production of an 11F capsule. This results in discrepant serotyping calls depending on the serotyping method used. Although the serological properties of the 33F-like capsule match that of the traditional serotype 33F, the 33F-like *cps* locus appears to be a mosaic of genes likely derived from other pneumococcal serotypes. This includes the presence of a *wcyO* acetyltransferase pseudogene, which has not been reported in serotype 33F previously. This work has important implications for improving the accuracy of serotyping methods used for measuring vaccine impact and invasive pneumococcal disease surveillance, particularly in LMICs, where pneumococcal serotype diversity is poorly understood.

60

Diversity – The Dilemma and The Joy of Diagnostic Veterinary Bacteriology

Pat Blackall[1]

1. University of Queensland, Brisbane, QLD, Australia

The diagnostic bacteriologist, be that in the clinical or the veterinary field, plays a critical role in providing the laboratory based skills that underpin disease diagnosis. The role is one that can bring great challenges but also great satisfaction. As both a front-line diagnostic bacteriologist and a researcher focussed on improving diagnostic technologies, I have had personal experience for over 40 years of those challenges and those satisfactions. Along with a plethora of highly skilled colleagues, I have had the good fortune to be involved with a number of projects that have included the recognition and naming of two new genera (*Avibacterium* and *Bibersteinia*) and six new species, the development of novel approaches to the classic serotyping of two major respiratory pathogens (*Avibacterium paragallinarum* and *Glaesserella parasuis*), the replacement of a traditional classic serotyping with modern molecular methods (*Actinobacillus pleuropneumoniae* and *Pasteurella multocida),* and the acceptance by the CLSI of a proposed methodology for antimicrobial resistance testing for a key pig pathogen (*Glaesserella parasuis*). Over those 40 years, the issue that has not changed is the unique diversity that faces the veterinary bacteriologist. A diagnostic clinical bacteriologist deals with one host (the human) while a veterinary bacteriologist deals with multiple diverse hosts. This host diversity then results in diversity in both the pathogens and the normal flora that confronts the diagnostic bacteriologist. In my very early days, the problem was that the breakthrough diagnostic technology (API 20E strips) had a data-base focussed on clinical bacteria and thus failed to recognise key veterinary pathogens. Over 40 years later, the key break through diagnostic technology (MALDI-TOF MS) has a significant drawback for veterinary bacteriologist –a data-base that does not reflect the diversity of pathogens and normal flora seen in a typical veterinary laboratory. "Plus ça change, plus c'est la même chose".

61

Bacterial Drivers of Neutrophil Behaviour During an *in vivo* Infection

Anton Peleg[1, 2]

1. Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, VIC, Australia

2. Infection and Immunity Program, Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Melbourne, VIC, Australia

Acinetobacter baumannii is one of the most significant hospital-acquired bacterial pathogens, able to cause a wide range of life-threatening infections and develop resistance to all currently available antibiotic agents. It is now the number one priority pathogen defined by the WHO as critically requiring research, discovery and development of new antibiotics. Our research focuses on the pathogenic mechanisms of *A. baumannii*, with the over-arching objective to identify novel therapeutic targets. We have established zebrafish as a model to study real-time interactions between innate immune cells and *A. baumannii* during infection. Innate immune cells such as neutrophils form a crucial first-line defence against bacterial pathogens, and we study bacterial-mediated factors that drive neutrophil migration to the site of infection. Through this work, we have identified a bacterial metabolic pathway that, when inhibited, leads to enhanced immune responses toward *A. baumannii*, improving bacterial clearance and reducing severity of disease. The enhanced immune response was secondary to accumulation of a metabolic by-product, which acted as a direct, bacterial-mediated attractant of neutrophils. These data identify a previously unknown mechanism of bacterial-guided neutrophil chemotaxis in vivo, providing insight into the role of bacterial metabolism in host innate immune evasion. These results also pave the way for novel therapeutic targeting of bacterial metabolism to stimulate immune responses to fight off infection.

Oral Abstracts

62

My journey with E. coli and urinary tract infections: 15 years, 3 continents, 6 universities and lots of fun on the way.

Makrina Totsika[1]

1. Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Kelvin Grove, Brisbane, QLD, Australia

In the space of my 15-year research journey, I have been studying *E. coli* causing urinary tract infections, a group of pathogens known as uropathogenic *E. coli* or UPEC. My studies have focused on UPEC virulence, adhesion mechanisms in particular, and my work has dissected their gene structure and regulation, unraveled their biogenesis and characterized their function. During this time, I have witnessed the global rise of UPEC to the top of WHO's pathogen priority list due to the emergence and rapid expansion of multidrug resistant clonal groups. Reports of pandrug resistant UPEC are increasing and the treatment of urinary tract infections is now a significant challenge for clinicians worldwide. The large number of urinary tract infections annually combined with the paucity in effective available treatments calls for immediate action. My lab investigates the molecular factors that confer UPEC fitness within a host and translates this knowledge into therapeutic strategies to overcome the problem of failing antibiotics. Our current work with different classes of virulence inhibitors is promising to provide effective and superior therapies for antibiotic-resistant urinary tract infections, as well as other common infections caused by different pathogens. This work could offer a paradigm-shift of how we treat infections in the near future.

63

Rapid Testing for Blood Cultures; Microbiology working with ID and Pharmacy to Maximize Patient Outcomes.

Susan Sharp[1]

1. Kaiser Permanente, Portland, OREGON, United States

The prompt initiation of antibiotics to treat infections has been proven to reduce morbidity and save lives. However, up to 50% of all antibiotics prescribed in acute care hospitals are either unnecessary or inappropriate. Antibiotics have potential serious side effects, including adverse drug reactions and *Clostridium difficile* infection. In addition, antimicrobial resistance can emerge from the inappropriate use of antibiotics leading to increased morbidity, mortality, and costs of health care. Prevention of the emergence of resistance and the dissemination of resistant microorganisms has the potential to reduce these adverse effects and their associated costs.

The appropriate use of antimicrobials is an essential part of patient safety and an effective antimicrobial stewardship program can limit the emergence and transmission of antimicrobial-resistant bacteria. In addition, antimicrobial stewardship can reduce health care costs without adversely impacting quality of care. This lecture will discuss different facets of an antimicrobial stewardship team as well as the role of the microbiology laboratory in the team. Practical examples of the impact of microbiology on antimicrobial stewardship, using blood cultures as an example, will be provided.

64

The machine comes to bacteriology: automation, agility and disruption.

Rod Givney[1]

1. NSW Health Pathology, NEWCASTLE, NSW, Australia

Automation is nothing new in microbiology. Any microbiological analysis using blood as a substrate and where the definitive diagnostic method is the identification of fragment of a molecule can readily be reduced to a test done using a commercial kit on an analyser in a clinical chemistry conveyor belt. Infectious diseases serology and, to some extent, clinical virology, automated a decade ago. Much of the work in these microbiological sub-specialities now consists of maintaining machines and the computerised assessment the quality of their diagnostic output, applying the standard methods familiar to any process of mass production.

Bacteriology and mycology testing have proved recalcitrant to automation. The late 20th century molecular revolution has failed to deliver on its promises for these fields. Partly this has been due to the variety of substrates but mainly because of the variety of species requiring detection, their genomic and antigenic complexity and the demand for antimicrobial susceptibility testing. However, overall specimen numbers have increased exponentially and high level managerial decisions from the 1990s on to reduce staff numbers and consolidate testing into fewer locations, necessitates more tests being done by fewer people but utilising the same space. In my particular location the response of senior management has been to experiment with automation using the COPAN-WASP and 24 hour services.

Oral Abstracts

65

A Molecular Gram-Stain for Sepsis: The Silent Killer

Flavia Huygens[1], RB Brandon[2]

1. Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University Of Technology, Kelvin Grove, QLD, Australia

2. Immunexpress Pty Ltd, Seattle, Washington, USA

Introduction

Sepsis is a leading cause of death worldwide; in the US it kills 250,000 people/yr and in Australia about 3000 people/yr . Sepsis can be difficult to distinguish from other non-infectious conditions that have similar clinical signs. Further, diagnosis of sepsis relies on blood cultures which are positive in only 10-30% of patients suspected of sepsis, and take at least 18 hours. The lack of sensitivity and timeliness of blood culture can lead to excessive use of broad-spectrum antibiotics in patients suspected of infection. It has been shown that most clinicians can make a correct narrow-spectrum antibiotic choice based on Gram-stain. We have developed a molecular Gram-stain assay that quickly and reliably detects and differentiates sepsis causing pathogens in whole blood and blood culture material based on Gram status.

Methods

SeptID® consists of a broad-range, 16S rDNA, multiplexed RT-qPCR capable of detecting and differentiating over 5400 bacteria based on Gram status, including most human pathogens. The assay relies on the use of two labelled probes, and one 16S rDNA Single Nucleotide Polymorphism, to differentiate Gram-positive from Gram-negative bacteria.We have previously demonstrated sensitivity of the assay using whole blood to be 1-10 cfu/mL. To determine SeptID® specificity we tested 225 positive and 100 negative blood culture samples (1mL). Gram status calls (Gram-positive, Gram-negative, mixed, negative) based on Ct values were made by two independent operators, and prior to any knowledge of clinical microbiology.

Results

Very high concordance was found for SeptID® results with clinical microbiology. Specifically, SeptID® and Gram-positive correlation was 97.99%; SeptID® and Gram-negative correlation was 98.61%. Further, SeptID® was able to determine whether a blood culture contained a mix of Gram-positive and Gram-negative organisms.

Conclusion

SeptID® can quickly, accurately and sensitively differentiate sepsis-causing pathogens based on Gram status. Nucleic acid based detection and differentiation of microbes in whole blood in patients suspected of sepsis holds promise for both decreasing time to diagnosis and aiding in antibiotic choice.

66

New tools for enhanced diagnosis and characterisation in atypical sepsis

Seweryn Bialasiewicz[2, 1], Tania Duarte[3], Claire Heney[4], Vichitra Sukumaran[4], Ross Balch[3], Son Nguyen[3], Lachlan Coin[3], Krispin Hajkowicz[5, 6]

1. Child Health Research Centre, The University of Queensland, South Brisbane, QLD, Australia

2. Children's Health Queensland, South Brisbane, QLD, Australia

3. Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD, Australia

4. Department of Microbiology, Pathology Queensland, Herston, QLD, Australia

5. Department of Microbiology, Royal Brisbane and Women's Hospital, Herston, QLD, Australia

6. School of Clinical Medicine, The University of Queensland, Herston, QLD, Australia

Introduction

Sepsis is a leading cause of death and disability in Australia. Microbiological diagnosis aiding appropriate antibiotic selection is critical in preventing rapid progression and death. For standard organisms, current culture-based diagnostics take greater than 48 hours, while fastidious or atypical organisms may take even longer, if they can be cultured at all. Every hour without appropriate treatment increases the risk of mortality in sepsis, thus rapid diagnostic approaches need to be considered, such as MinION nanopore real-time sequencing. Here we present the application of both the MinION sequencer and digital droplet PCR (ddPCR) to provide a rapid diagnosis and enhanced characterisation in a case of severe, atypical bacterial sepsis.

Methods

A woman with a history of a dog bite was admitted to the intensive care unit with severe sepsis, including multi-organ failure, severe acute kidney injury and haemolysis. EDTA whole blood samples were sent for MinION sequencing. The sample was sequenced overnight on the MinION using the rapid 1D library preparation protocol. A ddPCR assay was designed from the generated sequence reads to provide quantification of bacterial loads within the samples.

Results

The initial MinION run produced primarily human sequences, but also generated 19 reads of a combined length of approximately 20Kb which were identified as belonging to *Capnocytophaga canimorsus*, a fastidious, gram-negative rod which is typical commensal flora in dogs' mouths. Absolute quantification showed a 100-fold higher bacterial load in whole blood collected at presentation, compared to the plasma derived from the same sample, followed by a dramatic decrease in an EDTA collected 5 days later. In total, the time to diagnosis from obtaining the EDTA sample using the MinION was 20 hours, compared to over 5 days using traditional culturing methods. The result provided confidence in the selection of beta-lactam antibiotics and de-escalation of other classes.

Conclusion

New technologies can provide a supporting role to traditional techniques in improving and enhancing sepsis diagnosis to rationalise antibiotic therapy.

Oral Abstracts

67

Diagnostics and prognostics through machine learning; a tutorial and case study in gut microbiome-based weight-loss prediction

Mark N Read[1], Andrew J Holmes[1]

1. University of Sydney, Darlington, NSW, Australia

The implication of the gut microbiome in a wide variety of diseases has lead to its exploration as a biomarker for diagnostics and prognostics. However, single microbes rarely constitute reliable biomarkers, likely reflecting the microbiome's complexity and extensive cross-feeding. Rather, the biomarker patterns we seek are expressed across the entire community. Supervised machine learning (SML) techniques are an ideal approach through which to learn these patterns from supplied data, and serve as diagnostic and prognostic tools.

Yet, despite growth in the use of these tools, their operation and best-practice remain largely opaque to the Biological community. Educational material is lacking from Biological literature, and in Computer Science is reliant on ill-explained jargon and complex mathematics.

Our aim here is to present an overview of machine learning, accessible to Biologists. We cover what SML (both prediction and classification) is, and the conceptual differences between various prominent algorithms, e.g. random forests and neural networks. A common pitfall in machine learning is over-fitting, a phenomenon wherein a predictive model learns not only the trends in the supplied data, but its noise also. This harms subsequent post-learning performance on unseen data, for instance in the clinic, wherein the noise differs. We explore a best-practice pipeline, designed to avoid over-fitting, covering pipeline components such as feature selection and generation, cross validation, and the use of training-validation-test data sets. We explore the challenges that microbiome data poses for SML.

This exploration of machine learning is illustrated through a clinical weight-loss case study. People meet with varying success on any given dietary intervention, in part, we hypothesise, due to the microbiome. Obese participants were administered one of three diets: a high protein, Mediterranean, or low glycemic index diet. Faecal samples were taken prior to a three month dietary intervention, and weight-loss recorded thereafter. We predict weight-loss success on a given dietary intervention, aiming to tailor clinical strategy to the individual.

68

Phenotypic and genotypic characterisation of ribotype 251 strains of *Clostridium difficile* causing severe disease in the Australian community

Stacey Hong[1], Daniel R Knight[2], Melanie Hutton[3], Dena Lyras[3], Robert Carman[4], Thomas V Riley[5, 1, 2, 6]

1. School of Biomedical Sciences, University of Western Australia, Perth, Western Australia, Australia

2. School of Veterinary and Life Sciences, Murdoch University , Perth, Western Australia , Australia

3. Microbiology , Monash Biomedicine Discovery Institute, Melbourne, Victoria, Australia

4. TechLab Inc., Blacksburg, Virginia, United States of America

5. School of Medical and Health Sciences, Edith Cowan University , Perth, Western Australia, Australia

6. Microbiology, PathWest Laboratory Medicine, Perth, Western Australia , Australia

Background

Clostridium difficile infection (CDI) has reached an epidemic state in many developed countries with high incidence and severe disease. The binary toxin positive strain PCR ribotype (RT)027 has caused many outbreaks and remains highly prevalent in North America. This strain has never established in Australia, however, recently there have been reports of increased CDI incidence due to other strains that produce binary toxin. One such strain that has increased in prevalence Australia-wide is RT251. Herein, we genotypically and phenotypically characterised RT251 strains isolated in Australia and North America to ascertain virulence factors and the evolutionary history of this lineage.

Methods

C. difficile RT251 strains were isolated by toxigenic culture (n=124). Genetic characterisation was performed using toxin gene profiling, whole genome sequencing (WGS), *in silico* multilocus sequence typing (MLST) and core-genome single nucleotide variant (SNV) analyses. Antimicrobial resistance was determined using agar incorporation methods, *in vitro* toxin production was confirmed by Vero cell and HT-29 cytotoxicity assays. Motility assays were performed and pathogenicity was observed in a murine model on selected isolates.

Results

WGS and MLST clustered RT251 in the same evolutionary clade (clade 2) as RT027. Core-genome analyses revealed RT251 strains from Australia were highly clonal, separated by <10 SNVs. All Australian strains were motile and shared a common ancestor with a strain from Virginia, USA, isolated in 2012 (2-7 SNVs), indicating this lineage was of North American origin. All isolates were susceptible to metronidazole and vancomycin; one showed clindamycin and erythromycin resistance. Despite comparatively lower levels of *in vitro* toxin production, RT251 infection resembled infection with epidemic RT027 *in vivo*. Mice showed marked weight loss, severe disease within 48h post-infection and required euthanising.

Conclusion

Our findings indicate Australian *C. difficile* RT251 strains share a common ancestry with RT027 and are likely of North American origin. How RT251 entered Australia requires further investigation, however, these data emphasise the importance of ongoing surveillance for new strains of *C. difficile*.

69

Rapid Laboratory responses to ceftriaxone-resistant *Neisseria gonorrhoeae*; on behalf of the National Neisseria Network, Australia.

Ella Trembizki[1], Amy Jennison[2], Graeme Nimmo[3], Monica Lahra[4, 5], David Whiley[1, 3]

1. The university of Queensland Centre for Clinical Research (UQCCR), Brisbane, QLD, Australia

2. Public Health Microbiology, Queensland Health Forensic and Scientific Services, Brisbane, Queensland, Australia

3. Pathology Queensland , Brisbane, Queensland

4. New South Wales Health Pathology Microbiology, WHO Collaborating Centre for STD, Randwick, New South Wales, Australia

5. School of Medical Sciences, The University of New South Wales, Sydney, New South Wales, Australia

Background: Ceftriaxone and azithromycin dual therapy is the recommended treatment for gonorrhoea in most settings. In the recent months, there have been four clinical isolates of *N. gonorrhoeae* detected resistant to ceftriaxone, two of which had high level resistance to azithromycin. We characterised the isolates via whole genome sequencing (WGS) and developed a rapid PCR method for direct screening of clinical samples to detect the ceftriaxone resistance mechanism .

Methods: WGS and bioinformatics analysis for in silico genotyping, resistance genes and core genome MLST was conducted at the Queensland Health Forensic and Scientific Services to assess strain relatedness and to identify mechanisms of resistance. We used these sequencing data to develop a real-time PCR assay to detect the characteristic alterations associated with ceftriaxone resistance in these four strains. Following validation, the assay was applied *to N. gonorrhoeae*-positive PCR clinical samples from Queensland, and NSW collected in 2018 (n=approx. 800 and ongoing, provided by Pathology Queensland and the WHO CC for STD, NSW).

Results: WGS determined that the four isolates harboured the same ceftriaxone resistance mechanism, involving specific alteration of a 'type 60' *penA* allele. The four isolates comprised two different MLST types, ST1903 and ST12039, with the latter ST12039 isolates also harbouring the *23S* gene A2059G alteration conferring high-level resistance to azithromycin. All clinical samples screened with the real-time PCR assay for the year 2018 have so far provided negative results suggesting no further spread in Queensland at this stage.

Discussion: These rapid responses to new incursions of antibiotic resistant organisms of public health concern to provide enhanced and timely information for surveillance and enable enhanced detection and to determine spread. The NNN continues to perform enhanced testing across jurisdictions as part of the outbreak investigation.

70

Transposon maker IS*26*, flagship of the versatile IS*6* family of insertion sequences

Christopher J Harmer, Carol Pong, Ruth M Hall

In Gram-negative bacteria, the insertion sequence IS*26* plays a major role in disseminating antibiotic resistance genes via the formation of compound transposons. Despite this, and despite being discovered over three decades ago, until recently IS*26* was relatively neglected. Over the past five years we have shown that in addition to the well-known replicative cointegration mode, IS*26* can use a conservative, self-targeted reaction to form a cointegrate between two DNA molecules that each contain a copy of the IS. This occurs in preference to the replicative mode when two IS*26* are present. During the conservative reaction, the strand exchange(s) occur at the same end of the IS, but can occur at equal efficiency at either IS end. In the products of the conservative reaction, the two IS*26* are always in direct orientation, resembling a traditional class 1 compound transposon, and this mechanism has been used to introduce new genes *in vitro*.

To determine the functional characteristics of the IS*26* transposase (Tnp26) and to better understand the interactions between IS*26* and Tnp26, we computationally modelled the protein. We have subdivided and investigated several putative domains, including a helix-helix-turn-helix DNA binding domain, the DDE catalytic core, and a potential dimerization domain at the extreme N-terminus. Site-directed mutagenesis was used to introduce amino acid substitutions into conserved residues, and their importance in transposase activity was determined. A natural variant of IS*26* that carries an amino acid substitution in the DDE catalytic core is over-represented in intramolecular inversion and deletion events was also examined. This substitution was shown to significantly increase transposase activity.

The ability of Tnp26 to function in either a replicative or conservative mode is likely to explain the prominence of IS*26*-bounded transposons in the resistance regions found in Gram-negative bacteria. IS*26* relatives that play important roles in the mobilization of resistance genes in Gram-positive bacteria will need to be re-examined to determine if they too can utilise this new mechanism.

71

Epidemiology, mechanisms and implications of colistin resistance in *Klebsiella pneumoniae.*

Timothy Kidd[1]

1. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

The recent widespread emergence of multidrug–resistant and extensively-drug-resistant *Gram-negative bacteria* is an important public health challenge worldwide. Confronted with diminishing therapeutic options, colistin (polymyxin E) is increasingly being used by clinicians as a last-line agent for treating infections with these important pathogens. Colistin is a potent cationic peptide which interacts with the anionic lipid A moiety of the Gram-negative lipopolysaccharide structure to promote membrane permeability, cellular leakage and subsequent bacterial death. However, the renewed clinical use of this agent, coupled with its widespread agricultural use in some countries has driven the rapid dissemination of *K. pneumoniae* strains showing resistance to colistin. Here we will discuss the recent research findings from our group and elsewhere to: i) highlight the global epidemiology and evolution of colistin resistance in *K. pneumoniae*, ii) outline the molecular mechanisms involved in both chromosomal- and plasmid-mediated *K. pneumoniae* colistin resistance, and iii) consider the implications for treatment outcomes and future surveillance strategies.

72

Alarming Expansion of Penicillin-resistant Serogroup W *Neisseria meningitidis* and Identification of a borderline-ceftriaxone-susceptible Strain in Western Australia

Shakeel Mowlaboccus[2, 1], Keith A Jolley[3], James E Bray[3], Geoffrey W Coombs[4], Jane D Bew[5], David J Speers[5, 6], Anthony D Keil[7], Charlene M Kahler[2, 8, 1]

1. School of Biomedical Sciences, The University of Western Australia, Perth, Western Australia, Australia

2. Marshall Center for Infectious Diseases Research and Training, The University of Western Australia, Perth

3. Department of Zoology, University of Oxford, Oxford, UK

4. School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia

5. Department of Microbiology, PathWest Laboratory Medicine, Perth

6. School of Medicine and Pharmacology, The University of Western Australia, Perth, Western Australia, Australia

7. Department of Microbiology, PathWest Laboratory Medicine WA Princess Margaret Hospital for Children, Perth, Western Australia, Australia

8. Telethon Kids Institute, Perth, Western Australia, Australia

Introduction

Neisseria meningitidis (meningococcus) causes invasive meningococcal disease (IMD) which has a mortality rate of 6%. Disease is predominantly caused by serogroups A, B, C, W, X or Y. Multi-locus sequence typing (MLST) classifies meningococci into sequence types (ST) and clonal complexes (cc). Recent global outbreaks have been caused by meningococcal serogroup W (MenW) belonging to the cc11 lineage. MenW has become the predominant cause of IMD in Australia since 2016.

Objective

The aim of this study was to analyse the whole-genome sequences of invasive MenW:cc11 from Western Australia (WA) and investigate changes in antibiotic susceptibility.

Methods

Genomic DNA of 33 MenW:cc11 strains isolated from patients in WA were sequenced using Illumina paired-ends. Raw reads were assembled and curated using the BIGSdb genomics platform from the PubMLST database.

Results

In WA, the first MenW:cc11 case appeared in 2013. This was followed by two cases in 2014, three cases in 2015, 13 cases in 2016 and 14 cases in 2017. In this collection, six different STs were identified – ST-11 (n=17), ST-1287 (n=3), ST-3298 (n=1) and ST-12351 (n=10), ST-13125 (n=1) and ST-13135 (n=1). Resistance to ciprofloxacin or rifampicin was not identified. However, variation in penicillin susceptibility was observed: 10 isolates showed high susceptibility (MIC=0.064 mg/L) and 23 isolates were resistant (0.25–0.5 mg/L). Core genome phylogeny identified two main clusters, A and B. All penicillin-susceptible isolates fell in Cluster A and possessed the *penA*_59 allele. The penicillin-resistant meningococci, all isolated in 2016 and 2017, fell in cluster B and possessed the *penA*_253 allele. Exchange of p*enA*_59 for *penA*_253 in Cluster A isolates resulted in a significant increase in penicillin MIC. Finally, one outlier strain isolated from a traveler encoded a novel *penA* allele. This allele conferred reduced susceptibility to penicillin and to the extended-spectrum cephalosporin ceftriaxone.

Conclusions

In conclusion, core-genome analysis identified the emergence of a new cluster of penicillin-resistant MenW:cc11 in WA in 2016 which is currently expanding alarmingly and may thus impact treatment regimens internationally.

73

OMV-mediated horizontal gene transfer occurs in Gram negative bacteria.

Ella Johnston, Natalie Bitto, Steve Petrovski, Maria Liaskos

Gram negative bacteria produce nanoparticles known as outer membrane vesicles (OMVs) that contain bacterial products including DNA, proteins and lipids. Due to the ability of OMVs to package DNA within them, it is suggested that they are able to facilitate the transfer of genes between bacterial species. This study examines the ability of OMVs isolated from Gram negative *Pseudomonas aeruginosa* to package and transfer DNA to other bacteria.

In this study, OMVs were isolated from *P. aeruginosa* PAO9505 harbouring a plasmid encoding for antibiotic resistance. Examination of these OMVs using DNA staining revealed that DNA was associated on the outside, in addition to being contained within OMVs. In order to confirm that plasmid DNA was contained within OMVs, vesicles were treated with DNase and PCR was used to confirm the presence of intact plasmid DNA pre- and post-treatment. Plasmid-containing OMVs were used to transform recipient *P. aeruginosa* PAO9503, resulting in the generation of antibiotic resistant transformants. Moreover, as bacteria grown in conditions of stress increase their OMV production and alter their cargo composition, we investigated the ability of antibiotic stress to alter the amount of DNA contained within OMVs and their subsequent transformation efficiency. To do this, OMVs were isolated from *P. aeruginosa* exposed to a sublethal amount of gentamicin, and OMV production was quantified by NanoSight nanoparticle tracking analysis. We identified that antibiotic treatment of bacteria increased OMV production 3 fold, while decreasing their DNA content when compared to control OMVs. We

are currently investigating the effect of sustained antibiotic treatment on altering the biogenesis of OMVs, their DNA composition and their ability to transfer DNA between bacteria.

Collectively these findings identify that OMVs are a mechanism used by bacteria to transfer DNA encoding for antibiotic resistance to other bacteria, and that stress affects the packaging of nucleic acids into OMVs. The outcomes of this study suggest that OMV-mediated DNA transfer may contribute to the spread of antibiotic resistance between bacterial strains.

74

A novel TA system provides plasmid stability and antibiotic tolerance in Enterobacteriaceae

Muhammad Kamruzzaman[1], Jonathan Iredell[1, 2]

1. The Westmead Institute for Medical Research, The University of Sydney, Westmead, NSW, Australia

2. Westmead Hospital, Westmead, NSW, Australia

Toxin-antitoxin (TA) systems were initially discovered as plasmid addiction systems on low-copy-number plasmids through post-segregational killing. Thousands of TA loci have since been identified on chromosomes, plasmids and mobile elements in bacteria and archaea and have received increasing attention due to their diverse role in bacterial physiology and in maintenance of genetic elements. In this study, we identified and characterised a plasmid mediated type II TA system in Enterobacteriaceae. Protein sequence identity, secondary structure and conserved domain database search identified it as a member of the ParDE super family. This TA system is distributed in the IncI and IncF type plasmids of *E. coli* and other Enterobacteriaceae. Like the RK2 plasmid ParDE system, this novel systems (herein, ParDE^{I}) inhibits bacterial growth and exhibits cell elongation upon toxin activation and functions as a plasmid addiction system in *E. coli*. Expression of this TA system increased 2-4 fold upon exposure to sub-minimum inhibitory concentration (MIC) doses of aminoglycoside, quinolone and beta-lactam antibiotics and ParDE^{I} encodes tolerance of supra-MIC doses, with increased persister cell formation by ~100-1000 fold in *E. coli*. Additionally, this TA system provides a survival advantage in heat stress and the ParE^{I} toxin promotes biofilm formation in *E. coli*. This novel plasmid addiction system increases persister cell and biofilm formation and promotes antibiotic and (heat) stress tolerance, and is likely to be an important factor in the success of IncI and IncF antibiotic resistance plasmids that carry it.

75

Infectious diseases in Australian wildlife – recent studies performed in partnership with wildlife veterinarians

Joanne Devlin[1]

1. The Melbourne Veterinary School , The University of Melbourne, Parkville, Victoria, Australia

Australian wildlife can be hosts to a variety of different infectious agents that are of significance to the wildlife themselves, or of significance to other animals or to people who are in contact with wildlife. Recently we have studied pathogens in a range of different wildlife species, particularly native birds and marsupials. Many of these studies are in collaboration with veterinarians from Zoos Victoria, or with other wildlife veterinarians. Some of our most recent work has focussed on pathogens (*Salmonella*, *Campylobacter*, *Chlamydia*, herpesviruses and other viruses) present in Australian native birds presenting to Healesville Sanctuary, as well as pathogens (*Chlamydia*, Koala Retrovirus, herpesviruses) present in different populations of Victorian koalas. Ongoing work is focussed on zoonotic and other pathogens present in possums in Melbourne and surrounds, as well infectious agents in bent-wing bats. This work helps to characterise risks to wildlife and also to veterinarians, wildlife carers and other people who are in contact with wildlife. Our work has revealed that most zoonotic pathogens are present at a low prevalence but other pathogens are present at a very high prevalence. Examining the phylogenetic relationships between pathogens present in wildlife and other species provides information about potential transmission events. Our work has revealed evidence supporting complex interspecies transmission of beak and feather disease virus in Australian birds, and evidence supporting livestock-wildlife transmission of *Chlamydia pecorum* in Victorian koalas. Understanding the transmission of these pathogens ultimately facilitates the implementation of appropriate control measures.

76

Zoonotic bacterial pathogens

Conny Turni[1]

1. University of Queensland, Dutton Park, QLD, Australia

The transmission of bacterial zoonotic pathogens from animals to humans can occur in several ways: - via inhalation or ingestion, via the conjunctiva or via bites and other injuries inflicted by animals. The literature suggests that for transmission to occur from animal to humans a degree of proximity is required or, as in most cases, contact needs to occur, as it is believed aerosol transmission occurs only over very short distances.

One pathogen commonly transmitted to humans via bite is *Pasteurella multocida*, a bacterium known to cause numerous endemic and epizootic diseases in a wide range of domestic and wild animal species. *P. multocida* is part of the normal flora in the upper respiratory tract of many animal species from wild birds, domestic animals, marine mammals to chimpanzee, but is also both an opportunistic and a primary pathogen. The disease symptoms observed depend on the mode of transmission, on the host species and on the strain of *P. multocida*. Transmission to humans has been reported due to bites, scratches and licks from wild and domestic animals. Our research group has researched unusual intra- species transmissions of *P. multocida* and we have been involved in cases of zoonotic infections associated with close relatives of *P. multocida – Lonepinella* spp and *Mannheimia glucosida*.

The worrying point is that transmission of these pathogens does not have to be due to bites or injuries. Rather, contact with abrasions of skin via licking or contaminated environment is enough. This indicates that health and safety issues are an important aspect when handling animals, be they wild or domestic animals.

Oral Abstracts

Importantly, transmission is not only in one direction and can also involve transmission of disease from humans to animals, reverse zoonosis. Of particular importance, due to the link to antimicrobial resistance, is reverse zoonosis associated with methicillin-resistant *Staphylococcus aureus* (MRSA). It has been shown that strains of MRSA can be transmitted from humans to animals and vice versa.

77

Equine chlamydiosis: emerging threat or under-diagnosed disease?

Cheryl Jenkins[1], Martina Jelocnik[2], Joan Carrick[3], Adam Polkinghorne[2]

1. NSW Department of Primary Industries, Menangle, NSW, Australia

2. University of Sunshine Coast, Sippy Downs, Qld, Australia

3. Equine Specialist Consulting, Scone, NSW, Australia

Chlamydia psittaci is a broad host range pathogen with avian hosts acting as major reservoirs. Zoonoses are acquired via inhalation and are usually linked to contact with birds or contaminated substrates. *C. psittaci* infections in mammalian species other than humans have been less well-studied with prevalence rates potentially underestimated. In Australia, cases of equine reproductive loss have recently come under the spotlight due to apparent zoonotic transmission of *C. psittaci* from equine placental membranes to humans, a previously unrecognised route of transmission for this organism. We investigated the role and genetic identity of *C. psittaci* strains involved in an epizootic of equine reproductive loss and associated zoonoses in New South Wales using molecular and genomic approaches. A total of 199 cases of equine reproductive loss from 55 properties across NSW were examined. Placental and foetal swabs were tested for *C. psittaci* using real-time PCR to determine infection prevalence. To determine whether *C. psittaci* was likely to be responsible for equine reproductive loss, tissue samples were examined by *in situ* hybridisation, and chlamydial loads were also determined by qPCR. Finally, genome sequencing was undertaken from clinical samples to determine the strain of *C. psittaci* associated with equine abortion and associated zoonoses. The prevalence of *C. psittaci* infection detected in this study across all cases on all properties was 21.6%. *C. psittaci* was detected at 21 out of 55 properties sampled and found to be clustered around Scone and Wagga Wagga. Significantly more properties in the Scone area had *C. psittaci* positive cases compared to those outside that area, suggesting an abortion cluster. Genome sequencing and MLST typing of isolates from Scone and Wagga Wagga suggested that equine *C. psittaci* isolates were clonal and belonged to the globally distributed 6BC cluster known to be associated with Australian psittacines. *In situ* hybridisation confirmed the presence *C. psittaci* within equine tissues and the median load of *C. psittaci* was significantly higher in placental membranes compared to foetal tissues ($P < 0.01$). High chlamydial loads ($> 1 \times 10^6$ organisms/mg of tissue) were detected in samples collected during this study, including those from the zoonosis-linked index case. These results suggest that equine chlamydiosis may have resulted from spillover of infection from native parrots, that *C. psittaci* likely plays a role in equine reproductive loss and that infected foetal membranes frequently carry a sufficient load of *C. psittaci* to pose a potential risk to human health.

78

Fowl Cholera; a modern insight into an old disease.

Lida Omaleki[1], Brian M. Forde[2], Conny Turni[1], Thom P. Cuddihy[3], Pat J. Blackall[2], Scott A. Beatson[1]

1. Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Queensland, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland, Australia

3. QFAB Bioinformatics, Research Compute Centre, The University of Queensland, St Lucia, Queensland, Australia

Fowl cholera caused by *Pasteurella multocida* continues to be a problem in meat chicken breeder operations and has emerged in organic meat chicken and free-range layer production systems as well.

Lipopolysaccharide is one of the most important immunogenic virulence factors of *P. multocida*. Recent works have demonstrated that killed vaccines give protection only against strains with identical or nearly identical lipopolysaccharide (LPS) structures. As well, *P. multocida* strains of LPS genotype 3, identified by PCR, can have a range of truncated lipopolysaccharides.

Here we use whole genome sequencing and phylogenomic analysis to investigate isolate relatedness during outbreaks of fowl cholera over the years within two free-range poultry farms. A total of 125 *P. multocida* isolates were collected from two unrelated farms, one organic meat chicken and one free-range layer farm during cholera outbreaks. Whole genome sequencing data has been also used to predict the LPS outer core biosynthesis locus type and the multi locus sequence typing (MLST) of the isolates.

In silico LPS typing identified that the majority of isolates on both farms were carrying LPS type 3 with a small number of isolates on the meat chicken farm characterised as carrying LPS type 1. Sequence comparisons revealed that LPS outer core biosynthesis locus of the isolates carrying LPS type 1 were highly conserved. In contrast, the LPS locus of type 3 isolates were found to be highly diverse, which complicates selection of an appropriate and effective vaccine.

Core genome SNP tree demonstrated that isolates within each outbreak are highly related to each other. Moreover, different clones of *P. multocida* have been responsible for fowl cholera outbreaks over time. We also demonstrated that different clones of *P. multocida* have the capacity of carrying LPS type 3. The capacity of using genomics data to predict the structure of LPS type 3 will be further discussed

Our study clearly demonstrates that *in silico* LPS and MLST typing are suitable substitution for older genomics methods.

This project is co-funded by AgriFutures Australia and Australian Eggs.

Oral Abstracts

79

Multiple bacterial veterinary pathogens contain phase-variable regulons; phasevarions

John Atack[1], Pat Blackall[2], Michael Jennings[1]

1. Griffith University, Gold Coast, QLD, Australia

2. QAAFI, The University of Queensland, Brisbane, Queensland, Australia

Many bacterial pathogens contain randomly-switching methyltransferases that control phase-variable regulons – phasevarions. All current examples control expression of genes involved in pathogenesis and host-adaptation, and many regulate putative and current vaccine candidates. Effective vaccines require stably expressed targets; individual phase-variable can be identified *in silico* as they contain easily recognised features, but genes controlled by phasevarions do not, complicating the rational design of vaccines. We have identified and begun to study phasevarions controlled by the switching of both Type I and Type III methyltransferases in several important bacterial veterinary pathogens: Streptococcus suis and Actinobacillus pleuropneumoniae are major swine pathogens, with S. suis also a major cause of bacterial meningitis in humans, particularly in S.E. Asia; Mannheimia haemolytica is a major bovine pathogen. All three organisms contain randomly switching methyltransferases, with all three pathogens containing both Type I and Type III methyltransferases that are able to phasevary – a phenomenon never before observed. Pacific Biosciences SMRT sequencing and methylome analysis of the methyltransferases from these organisms has deciphered their specificity, and demonstrated that different genes/alleles methylate different target sequences, and therefore control different phasevarions. Analysis of the protein profiles of strains containing phase-variable methyltransferases shows protein expression differences correlating with methyltransferase switching.

Our analysis shows that phasevarions are present in diverse veterinary pathogens, and need to be characterised in order to identify the stably expressed protein repertoire of these organisms. This work will provide a framework for the rational design of vaccines and treatments against these bacteria.

80

Campylobacter transmission in Australian free-range broiler flocks

Pongthorn Pumtang-on[1], Tim Mahony[2], Rod Hill[1], Thiru Vanniasinkam[1]

1. School of Biomedical Science, Charles Sturt University, Wagga Wagga, NSW, Australia

2. Centre for Animal Science, Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia

Chicken meat is regarded as a major source of *Campylobacter* infection in humans. Understanding the dynamics of *Campylobacter* transmission in chicken flocks could provide the knowledge required to develop effective interventions for *Campylobacter* control at the farm level. However, information on the sources and timing of *Campylobacter* colonisation of Australian free-range chicken farms is limited. This study aimed to investigate the source(s) of *Campylobacter jejuni* and *C. coli* colonisation and transmission in free-range broiler chickens in New South Wales, Australia. Samples from broiler farms and breeder farms were obtained and tested using culture, mass spectroscopy and multiplex qPCR. The results of this study showed that *C. jejuni* is the predominant species in broiler flocks. *C. jejuni* and/or *C. coli* were detected in the farm environment before chick placement in two flocks and *C. coli* was detected in one broiler flock when chicks were 10 days old. The highly variable *flaA* gene of *Campylobacter* was selected for genotyping using a High-Resolution Melt PCR (HRM-PCR). All *C. jejuni* and *C. coli* genotypes isolated from broiler flocks were further characterised by Multilocus Sequence Typing (MLST). Using these methods nine genotypes of *C. jejuni* and five genotypes of *C. coli* were identified. Two *C. jejuni* genotypes (ST-257 complex and ST-45 complex) were identified as the predominant genotypes. While, *flaA* allele no 36 was the predominant *C. coli* genotype. The same *C. jejuni* and *C. coli* genotypes isolated from broiler flocks were found in the farm environment such as pest faeces, soils, sheds, boots and drinking water. These results suggest that horizontal transmission played an important role in *C. jejuni* and *C. coli* colonisation of broiler flocks in this study. There was minimal evidence of vertical transmission between breeder and broiler flocks. These results suggest that any intervention to reduce *Campylobacter* colonisation of chickens would need to be applied on broiler farms.

81

Modulation of antiviral responses by low dose-DNA damaging agents

Michael P Gantier[1], Genevieve Pepin[1]

1. Hudson Institute of Medical Research, Clayton, VIC, Australia

Topoisomerase I (top1) inhibition relying on low-dose Camptothecin (CPT) has recently been proposed to show interesting therapeutic potential to dampen cytokine storms associated with high morbidity upon viral infections (Science, 2016 352(6289):aad7993), for its capacity to block transcription of pro-inflammatory factors. Critically, CPT also impairs the DNA repair machinery and results in DNA damage. In light of accumulating evidence demonstrating that nuclear DNA damage can result in antiviral activation through engagement of the cytosolic cGAS-STING pathway, we investigated the effect of low-dose CPT on innate immune responses (1). We discovered that minor DNA damage from top1 inhibition with low-dose CPT could trigger a strong antiviral immune response through cyclic GMP–AMP synthase (cGAS) detection of cytoplasmic DNA, but that this was dependent on the presence of viral oncogenes. Conversely, dual top1/2 inhibition by acriflavine (2) could result in antiviral effects independent of such viral oncogenes. Our work demonstrates that the mechanism of how DNA damage engages antiviral responses is more complex than previously proposed, and calls for caution in the therapeutic use of low-dose chemotherapy agents to modulate antiviral responses in humans.

1. Pépin G, Nejad C, Ferrand J, Thomas BJ, Stunden HJ, Sanij E, Foo CH, Stewart CR, Cain JE, Bardin PG, Williams BRG, Gantier MP. Topoisomerase 1 Inhibition Promotes Cyclic GMP-AMP Synthase-Dependent Antiviral Responses. MBio. 2017 8(5). pii: e01611-17. doi: 10.1128/mBio.01611-17.

2. Pépin G, Nejad C, Thomas BJ, Ferrand J, McArthur K, Bardin PG, Williams BR, Gantier MP. Activation of cGAS-dependent antiviral responses by DNA intercalating agents. Nucleic Acids Res. 2017 45(1):198-205. doi: 10.1093/nar/gkw878.

Oral Abstracts

82

Vaginal Microbiota and HIV Susceptibility

Gilda Tachedjian

There is increasing evidence that HIV transmission can be influenced by the vaginal microbiota. In women of reproductive age, optimal vaginal microbiota associated with protection against HIV are typically dominated by beneficial *Lactobacillus* spp. In contrast “suboptimal” microbiota or vaginal dysbiosis is characterised by a dramatic depletion of vaginal lactobacilli and an increase in load and diversity of anaerobic bacteria. These highly diverse vaginal microbiota are prevalent in adolescent girls and young women in sub-Saharan Africa who are up to three times more likely to be infected with HIV compared to their male counterparts. Vaginal dysbiosis is associated with an increase in vaginal pH, breakdown of the protective mucosal barrier and subclinical genital inflammation, the latter resulting in the activation and recruitment of HIV target cells. In addition there is a dramatic depletion of a major microbiota organic acid metabolite, lactic acid. Here, studies will be presented demonstrating that lactic acid produced by the vaginal microbiota has microbicidal and virucidal activities that may protect against sexually transmitted infections and endogenous opportunistic bacteria as well as immune modulatory properties on the cervicovaginal epithelium that could decrease HIV susceptibility. These findings highlight the potential use of lactic acid containing agents in the lower female reproductive tract as adjuncts to female-initiated strategies to reduce HIV acquisition

83

Not available at time of printing

Andrew van den Hurk[1]

1. Queensland Health, Brisbane, QLD, Australia

Not available at time of printing

84

Novel delivery of a live-attenuated chikungunya virus vaccine candidate

Adam Taylor[1]

1. Griffith University, Southport, QLD, Australia

Chikungunya virus (CHIKV) is an alphavirus that causes outbreaks of chikungunya, a mosquito-borne viral disease. The disease causes highly debilitating arthritis that can persist for months or years. Alphaviruses related to CHIKV, e.g. Ross River virus (endemic in Australia & Pacific) cause similar incapacitating musculoskeletal disease. There are no CHIKV antivirals, nor is there a licensed vaccine. We have developed and a live-attenuated CHIKV vaccine candidate, CHIKV-NoLS. CHIKV-NoLS immunised mice are protected from CHIKV infection. Additionally, this vaccine shows cross-protection against Ross River virus. A live-attenuated vaccine is a desirable means of disease prevention due to ease of production, low cost and avoidance of multiple boosters. However, propagation of CHIKV-NoLS is limited by its attenuated replication. Removing this bottleneck in production would greatly benefit CHIKV-NoLS development. The investigation described here aims to bypass in vitro propagation by using a novel delivery method, allowing for *in vivo* inoculation. Results suggest delivery of CHIKV-NoLS using this methodology in mice elicits *de novo* CHIKV-NoLS particle production *in vivo*. Mice immunised using this strategy develop long-term protection from CHIKV disease.

85

miR-956 suppression delays viral pathogenicity in model *Drosophila melanogaster*-virus system

Verna M Monsanto-Hearne[1], Annabelle LY Tham[1], Zhee Sheen Wong[1], Sassan Asgari[1], Karyn N Johnson[1]

1. University of Queensland, St Lucia, ACT, Australia

The outcome of viral pathogenesis is determined by many biological components including small non-coding microRNAs (miRNAs). Studies have shown that miRNA binding to complementary transcript sequences alter the gene expression of host factors and subsequently impact the host-virus interaction. However, most experiments implicating miRNAs in viral pathogenesis are done *ex vivo* and/or using viruses which are not natural pathogens of hosts. Here, to investigate the *in vivo* role of miRNAs in natural host-virus association, the *Drosophila melanogaster*-Drosophila C virus (DCV) model system was used. *D. melanogaster* infection with the +ssRNA virus DCV induced differential abundance in miRNA levels, with the largest change observed for miR-956-3p. Loss of miR-956 in miR-956 knockout flies resulted to delayed viral accumulation and virus-induced mortality compared to wild-type flies, demonstrating that the observed decrease in miR-956 during virus infection has antiviral and host-protective consequences. miR-956 putative targets were identified and 84 of the targets were assayed for regulation upon miR-956 loss-of-function (LOF) and DCV infection. The virus-stimulated gene (VSG) Ectoderm-expressed 4 (*Ect4*) showed up-regulation upon miR-956 LOF and down-regulation upon DCV infection, suggesting miR-956 negative regulation of *Ect4* during viral infection. To analyse the role of *Ect4* regulation during DCV infection, *Ect4* knockdown flies were challenged with DCV. Inverse to the effects of miR-956 suppression, decrease in *Ect4* accelerated virus accumulation and virus-induced mortality. Take together, results show that, *in vivo,* the antiviral and host-protective consequences of miR-956 regulation during infection of the model *D. melanogaster* with its natural pathogen DCV is exerted through miR-956 modulation of the VSG *Ect4.*

86

Mapping the virome in wild-caught *Aedes aegypti* from Cairns and Bangkok

Martha Zakrzewski, Gordana Rasic, Jonathan Darbo, Lutz Krause, Yee S Poo, Igor Filipovic, Rhys Parry, Sassan Asgari, Greg Devine, Andreas Suhrbier

Medically important arboviruses such as dengue, Zika, and chikungunya viruses are primarily transmitted by the globally distributed mosquito *Aedes aegypti*. Increasing evidence suggests that transmission can be influenced by mosquito viromes. Herein RNA-Seq was used to characterize RNA metaviromes of wild-caught *Ae. aegypti* from Bangkok (Thailand) and from Cairns (Australia). The two mosquito populations showed a high degree of similarity in their viromes. BLAST searches of assembled contigs suggest up to 27 insect-specific viruses may infect *Ae. aegypti*, with up to 23 of these currently uncharacterized and up to 16 infecting mosquitoes from both Cairns and Bangkok. Three characterized viruses dominated, Phasi Charoen-like virus, Humaita-Tubiacanga virus and Cell fusing agent virus, and comparisons with other available RNA-Seq datasets suggested infection levels with these viruses may vary in laboratory-reared mosquitoes. As expected, mosquitoes from Bangkok showed higher mitochondrial diversity. Blood meal reads primarily mapped to human genes, with a small number also showing homology with rat/mouse and dog genes. These results highlight the wide spectrum of data that can be obtained from such RNA-Seq analyses, and suggests differing viromes may need to be considered in arbovirus vector competence studies.

87

Staphylococcus aureus follows a “DARC” path to lethal infections

Victor Torres[1]

1. NYU School of Medicine, New York, NEW YORK, United States

: The pathogenesis of *Staphylococcus aureus* is thought to depend on the production of pore-forming leukocidins that kill leukocytes and lyse erythrocytes. Two of these leukocidins, Leukocidin ED (LukED) and Hemolysin gamma AB (HlgAB), are necessary and sufficient to kill mice upon intravenous delivery in both infection and toxin challenge models. However, the mechanisms of lethality in these *in vivo* models are unknown. I will discuss our recent findings unraveling how LukED and HlgAB exhibit their lethal effect. Briefly, we discovered that these toxins cause vascular congestion and derangements in vascular fluid distribution that rapidly cause lethality. Surprisingly, the leukocidal and hemolytic functions of these toxins are dispensable for lethality. Instead, we identify the Duffy antigen receptor for chemokines (DARC) on nonhematopoietic cells as the critical target during lethal toxin challenge and bloodstream infection. We demonstrate that LukED and HlgAB target the vasculature and can directly injure primary human endothelial cells via DARC. Thus, endothelial cells are novel cellular targets for these leukocidins. The potential role of *S. aureus* leukocidins in manipulating the vascular dynamics of the host further highlights the importance of these virulence factors to *S. aureus* pathophysiology.

88

New lights on the phylogenetic and metabolic diversity of archaea and their key role in the evolution of life on Earth

Anja Spang[1, 2]

1. Royal Netherlands Institute for Sea Research, AB Den Burg (Texel), Netherlands

2. Department of Cell- and Molecular Biology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden

Archaea represent a major prokaryotic domain of life besides Bacteria but were recognized as such only about 40 years ago. Originally, members of the archaea were thought to be confined to extreme environments and of little ecological importance. However, recent advancements in sequencing technologies, which among others allows to sequence DNA directly from environmental samples or single cells using single cell and metagenomics methodologies has revealed the near ubiquitous presence of archaea in all studied environments on Earth. Furthermore, this wealth of sequencing data has unveiled that archaea are much more diverse than anticipated earlier and various novel lineages of high taxonomic rank were recently proposed. Importantly, detailed analyses of genomes of these novel archaea have provided fundamental new insights into the metabolic diversity and potential role of archaea in their natural habitats as well as into evolution of microbial life on Earth and the origin of Eukaryotes.

Here, I will present a historical perspective of the ‘evolution’ of the archaeal tree of life, which will give a synopsis of our current knowledge of archaeal phylogenetic and metabolic diversity today. Subsequently, I will discuss how the discovery of some of these lineages has allowed not only a changed perspective on the early evolution of Archaea but also on the role of archaea in the origin of eukaryotic cells and in the involvement in major biogeochemical nutrient cycles.

89

Act like a scientist! Using physical performance in undergraduate science teaching

Terry Mulhern[1], **Rinske Ginsberg**[2]

1. University of Melbourne, Parkville, VIC, Australia

2. Victorian College of the Arts, Faculty of Fine Arts and Music, University of Melbourne, Melbourne, VIC, Australia

Artistic creativity and scientific thinking are not mutually exclusive. Indeed, many scientists are highly creative and express this in their work and their personal lives. However, while the intersections between science and music or the visual arts are reasonably well explored, it seems that theatrical performance is the road less travelled.

It is clear from studies in primary and secondary education that well-considered and reflective use of theatrical performance can enhance and empower student learning in science (Ødegaard, 2003). So, why don’t we do this in higher education?

In 2017 we developed a teacher-centred physical performance which reconceptualised the structure, function and dynamics of the 20 naturally occurring amino acids. This interactive molecular ‘yoga’ session was presented live in class to over a thousand 2nd year Bachelor of Science and Bachelor of Biomedicine students. In 2018 we have flipped this idea and have initiated a trial of a student-centred activity we call *The Performing Sciences*. In *The Performing Sciences* students volunteer to script and present a short performance illustrating a concept from the curriculum with the surface goal of helping their classmates understand the concept better.

In addition to any impact on the audience, we have designed the activity so that participants should gain a deeper and more complete understanding of the topic they perform; discover new skills in verbal and nonverbal communication; develop the ability to deliver constructive criticism; and build confidence in public speaking and science communication.

1. Marianne Ødegaard (2003) Dramatic Science. A Critical Review of Drama in Science Education, Studies in Science Education, 39:1, 75-101.

90

Flipping the classroom: design, implementation and outcomes

Vincent Wheatley[1]

1. University of Queensland, St Lucia, QLD, Australia

In the flipped classroom approach, content is delivered outside the classroom and active learning opportunities are moved in, reversing the traditional lecture-homework format. This can lead to improved student engagement and outcomes, but there are a number of inter-linked elements to the practice that require careful design and implementation. To illustrate how this can be achieved, the design, implementation and outcomes of a flipped classroom approach in a large fluid dynamics course is presented as a case study. The instructional component is a SPOC (Small Private Online Course) consisting of video segments interspersed with online formative problem solving activities and assessment. Conceptual questions build up to complex numerical problems. To add value to the on-campus experience, traditional lectures are replaced with small group peer learning where we solve interesting, authentic problems straight from the coalface of industry and cutting edge research. Engaging with the type of problems students hope to take in their "dream jobs" takes students beyond the classroom and ignites their enthusiasm. With a class of over 250, we use digital response systems so groups can compare their results and receive timely feedback from the instructor and peers. The outcomes of this approach in terms of student engagement, learning and satisfaction over multiple cohorts will be presented.

91

Contract cheating in Australian higher education: Results from a nation-wide survey of students and staff

Tracey Bretag[1], **Rowena Harper**

1. University of South Australia, Adelaide, SA, Australia

In response to numerous media scandals which exposed student cheating in Australian universities as an issue of concern, the Australian Government Office for Learning and Teaching commissioned a research project to explore the relationship between contract cheating and assessment design. Based on over 14,000 responses from university students and over 1,100 responses from teaching staff, this keynote presentation will share the key findings from that project, including:

1. Contract cheating is influenced by three key factors: students’ dissatisfaction with the teaching and learning environment; students’ perception that there are ‘lots of opportunities to cheat’; and if students speak a Language Other than English (LOTE) at home.
2. Students ‘share’ their work a lot, and this can lead to cheating.
3. Students don’t care about contract cheating, and staff are not talking to them about it.
4. Suspected cases of contract cheating often aren’t reported, and when they are, the penalties are lenient.
5. Authentic assessment is not the ‘solution’. However, there are some types of assessment which are ‘less likely’ to be outsourced, yet these assessments are not widely used.

The presentation will provide a research-informed understanding of what contract cheating is, how and why it occurs, and the complex relationship between contract cheating and the teaching and learning environment. The presentation will briefly discuss practical strategies for addressing contract cheating via a multi-pronged approach which takes into consideration the broader higher education context, institutional frameworks, and what happens at the program and course levels.

Oral Abstracts

92

Eradication of Contagious Bovine Pleuropneumonia in Australia

Frances Morey[1]

1. Western Diagnostic Pathology, ALICE SPRINGS, NT, Australia

ERADICATION OF CONTAGIOUS BOVINE PLEUROPNEUMONIA IN AUSTRALIA

Contagious bovine pleuropneumonia is a disease that only affects bovine animals that was first recognised in Germany in 1693, and it is a disease that most of today's microbiologists will never have encountered. The causative organism is *Mycoplasma mycoides* subspecies *mycoides*. Susceptible cattle become infected by inhaling the droplets that are disseminated by the coughing of actively infected animals. The infected cattle may apparently recover, but approximately 25% will become carriers, and if stressed by adverse conditions will become highly infectious to any susceptible bovine host.

Pleuropneumonia first occurred in Australia in 1858, with the importation of five head of cattle from England, which included one infected heifer, onto a Victorian property This heifer died three weeks later, and the owner was advised he should destroy his entire herd – but the heifer had already infected a bullock team grazing on an adjoining property. Within a year the disease had spread rapidly via the overland road to New South Wales, and from there it spread into Queensland, across the north of Australia and later to Western Australia. Tasmania remained the only unaffected area of Australia. The worst affected area was in the north of Australia where in the 1930s 3 million cattle roamed over up to 5 million square kilometres which were mostly unfenced.

It took until 1973, using a modified test to screen cattle, that was developed in Alice Springs from the original standard test; and a cattle movement system developed by the Northern Territory Chief Veterinary Officer who had been an Army colonel using his military knowledge, to eradicate the damage done by one imported animal.

93

Veterinary Bacteriology in Queensland – a personal perspective

Pat J Blackall

Queensland (even ahead of the formation of the nation of Australia) had recognised the value of animal-based food industries and the sciences that supported those industries. In this talk, an overview of selected key highlights of veterinary microbiology in Queensland will be presented. The initial laboratory (the Stock Institute) was set up in 1893. The Queensland government had seen the success of the vaccination program for Cumberland disease – anthrax – in NSW sheep (a success that actually funded the construction of the Pasteur Institute). In a move that is very typical of even modern Queensland, the decision was made to "pinch" the best available microbiologist from the slower moving southern colonies – Adrien Lior (the producer of the Cumberland Disease vaccine). The bold move failed, as Lois Pasteur had ordered the return of his nephew after the insulting behaviour of the colonies in failing to award the rabbit control prize to Pasteur and his "cholera" bacillus. The alternative selection as laboratory head – Charles Pound –proved an inspired selection. Pound quickly developed a remarkably effective Institute (with various names and locations) that undertook pioneering work in cattle and poultry diseases. Pound remained in charge of the Institute until 1932. The glory days of Pound were replicated a number of years later under the direction of Geoffrey Clive Simmons and a team of outstanding microbiologists – particularly Michael (Des) Connole and Jean Elder. In this period, the Queensland laboratory worked on the recognition of new pathogens (*Actinobacillus seminis*), new serovars (*Leptospira* Serovar pomono) and the critically important V-4 variant of Newcastle Disease virus in chickens. While the number of veterinary laboratories in Queensland is now reduced to one, the work of that laboratory continues to support the animal and aquatic industries of Queensland.

94

Gram Stains to Genomics: Bacterial Identifications in a Public Health Laboratory

Jenny Davis

The four decades until the present day have seen major changes in the theory and practice of bacterial identification. Heightened expectations from the public, government authorities and clinicians – including accurate identifications of zoonotic bacteria, of potential bioterrorism agents, and of emerging pathogens – have raised the bar for both routine clinical microbiology laboratories and for the specialist identification laboratory. Accreditation and compliance requirements (particularly with Security Sensitive Biological Agents) have added complexity to laboratory test standards and documentation. Perhaps most significant are the changes in technology. Labour-intensive methods (dependent on experience and an "apprentice – style" learning) were streamlined into miniaturised and automated substrate based systems; then molecular biology and mass spectrometry transformed both bacterial taxonomy and laboratory practice. I will describe my experience of identification of unusual and "difficult" bacterial isolates in a public health laboratory over these years; I will give examples of particular diagnostic puzzles and problems; and I will illustrate my opinion that for me - while genomics is the current state-of-the-art technology – Hans Christian Gram's legacy remains a pillar of bacterial identification.

95

Reversing antibiotic resistance by destabilization of bacterial metal homeostasis

Mark Walker[1]

1. The University of Queensland, St. Lucia, QLD, Australia

The World Health Organization reports that antibiotic resistant pathogens represent an imminent global health disaster for the 21st Century. Gram-positive superbugs threaten to breach last-line antibiotic treatment, and the pharmaceutical industry antibiotic development pipeline is waning. Here we report the synergy between ionophore-induced physiological stress in Gram-positive bacteria and antibiotic treatment. PBT2 is a safe-for-human-use zinc ionophore that has progressed to Phase 2 clinical trials for Alzheimer's and Huntington's disease treatment. In combination with zinc, PBT2 exhibits antibacterial activity and disrupts cellular homeostasis in erythromycin-resistant group A *Streptococcus* (GAS), methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). We were unable to select for mutants resistant to PBT2+zinc treatment. While ineffective alone against resistant bacteria, several clinically relevant antibiotics act synergistically with PBT2+zinc to enhance killing of these Gram-positive pathogens. These data represent a new paradigm whereby disruption of bacterial metal homeostasis reverses antibiotic resistant phenotypes in a number of priority human bacterial pathogens.

96

New drugs from old bugs

Sacha J Pidot[1], **Marion Herisse**[1], **Torsten Seemann**[2], **Benjamin P Howden**[2], **Timothy P Stinear**[1]

1. University of Melbourne, Parkville, VIC, Australia

2. Microbiological Diagnostic Unit, The University of Melbourne, Melbourne, VIC, Australia

The development of new antibiotics is key to addressing the crisis in human health caused by the rise of multi-drug resistant superbugs. Empirical screening of bacteria and fungi for bioactive molecules has been the source of the most successful existing antibiotics. The most prolific producers of these metabolites are the Actinobacteria, particularly the genus *Streptomyces*, but high re-discovery rates amongst soil-derived organisms demand the testing of new reservoirs of biodiversity and bioactive molecules. Recent studies have shown that human-associated bacteria represent a previously untapped source of antimicrobial diversity. Here, we describe our experiences exploring the antimicrobial activity of a diverse culture collection of 700 human pathogenic Actinobacteria held by our state microbiology reference laboratory. We show that organisms from this collection produce compounds capable of inhibiting the growth of the hospital superbugs MRSA and VRE. To investigate the genetics behind the production of these antibiotics, we have sequenced 100 of these genomes, which has provided a glimpse into the immense secondary metabolic potential of these organisms. Furthermore, we have cloned and expressed several large gene clusters for the biosynthesis of predicted antibiotics in heterologous hosts, which is the first time this has been achieved using *Nocardia* species. Our investigation of this collection of strains, via a combination of molecular biology, genomics and chemical biology, paves the way for further discoveries capable of refilling the antibiotic pipeline.

97

Evaluation of novel inhibitors against the macrophage infectivity potentiator in Gram-negative bacteria

Jua Iwasaki[1], **Emily Kibble**[2, 1], **Nicole Bzdyl**[1], **Chen Ai Khoo**[3], **Geoff Coombs**[4], **Charlene Kahler**[1], **Anja Hasenkopf**[5], **Peter Myler**[6, 7], **David Fox**[6, 8], **Don Lorimer**[6, 8], **Hayley Newton**[3], **Ulrike Holzgrabe**[5], **Mitali Sarkar-Tyson**[1]

1. Marshall Centre for Infectious Diseases, School of Biomedical Sciences, University of Western Australia, Perth, Nedlands, WA, Australia

2. School of Veterinary and Life Sciences, Murdoch University, Perth, WA, Australia

3. Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia

4. School of Veterinary and Life Sciences, Murdoch University, Perth, WA, Australia

5. Institute of Pharmacy and Food Chemistry, University of Würzburg, Würzburg, Am Hubland, 97074, Germany

6. Seattle Structural Genomics Center for Infectious Disease (SSGCID), Seattle, Washington, USA

7. Center for Infectious Disease Research, Seattle, WA 98109, USA

8. Beryllium Discovery Corporation, Bainbridge Island, WA 98110, USA

The Macrophage infectivity potentiator (Mip) protein is a virulence factor encoded by Gram-negative intracellular pathogens. Mip proteins have been shown to have a virulence-associated peptidyl-prolyl isomerase (PPIase) activity that is important for bacterial survival and pathogenesis. Therefore, inhibition of Mip potentially represents a novel target for antimicrobial therapies towards Gram-negative bacterial pathogens. A group of pipecolic acid-derived small molecules have been shown to inhibit recombinant Mip from *Burkholderia pseudomallei* (Bps). Using a protease coupled PPIase assay, we have demonstrated that these compounds also have broad-spectrum inhibitory properties against the recombinant Mips from *Neisseria meningitidis* and *Coxiella burnetii*. Importantly, these inhibitors reduce the cytotoxic effects and intracellular survival of these pathogens in mammalian cell lines. Co-crystal studies of inhibitor-BpsMip complexes have aided in developing compounds with improved affinity and potency that exhibit enhanced *in vitro* activity against the Gram-negative bacteria investigated in these studies.

98

Rational design for therapeutic use of bacteriophages against pathogenic bacterial clones.

Carola Venturini, **Nouri Ben Zakour, Sandra Morales, Steven Branston, Zsuzsanna Kovach, Bethany Bowring, Nicholas Thomson, Jon Iredell**

In this era of global rapid spread of multiple antibiotic resistance in clinically relevant bacterial species, alternative or adjuvant therapies to antibiotics are urgently needed to both combat refractory infections and limit dissemination of problematic opportunistic pathogens [1]. Bacteriophage therapy may provide a valid clinical alternative, as obligately lytic bacteriophages can be readily isolated and have the potential to be effective against multidrug resistant bacteria [2]. However, routine implementation of bacteriophage treatment in the clinic is hindered by poor understanding of therapeutic applicability, penetration, and resistance issues [3]. We are currently investigating a rational design approach to the development of bacteriophage therapeutic cocktails to be used against pathogenic bacterial clones. The targets chosen for this work are ST131 *E. coli*, with $bla_{CTX\text{-}M\text{-}15}$, and cc258 *K. pneumoniae*, carrying bla_{KPC}, both globally disseminated highly virulent clones dominating the epidemiology of life-threatening multidrug resistant nosocomial infections [4]. These species are also common gut colonizers able to persist asymptomatically for up to 12 months, thus increasing the risk of transmission to vulnerable individuals [5]. We have fully characterized sets of target bacteria (60 ST131 *E. coli*; 20 cc258 *K. pneumoniae*), and tested the infectivity of >30 bacteriophages (AmpliPhi Biosciences Corporation) from our extensive libraries. Phages specific to each target population (n=8 for *E. coli*; n=12 for K. *pneumoniae*) were selected for detailed characterization. Using a combined genomic and molecular microbiology approach, we found that

the genetic diversity of each bacterial population was associated with specific phage susceptibility profiles reflecting the structural specificity of the bacterial outer cell envelope. This represents the first step in our workflow towards the establishment of a robust scientific protocol for the selection of phages for the development of optimal therapeutic cocktails.

1. WHO (2014) Antibiotic Resistance: Global report on surveillance 2014.
2. Lin et al (2017) World J Gastrointest Pharmacol Ther 8(3):162-173.
3. Abedon (2018) in Medina & López-Baena (eds) Host-Pathogen Interactions. Methods in Molecular Biology, vol. 1734. Humana Press NY.
4. Woodford et al (2011) FEMS Microbiol Rev 35(5): 736-755.
5. Kang et al (2005) Antimicrob Agents Chemother 49(2): 760-766.

99

Alternatives in tackling enzyme mediated antimicrobial resistance

Brinda Chandar, Debdutta Bhattacharya

The evolution of β-lactamases has led to threatening levels of enzyme mediated antibiotic resistance. The raise in the usage of antibiotics has decreased its effectiveness and progressed into untreatable infections. The identification of highly potential enzymes, New Delhi Metallo-beta-lactamase (NDM-1) and Extended spectrum β-lactamase (ESBL)are a menace to the public health as globally there are no clinically tested antibiotics available so far to treat such infections. Spectrum of efforts is made to combat enzyme mediated resistance by gram negative bacteria. However, very little has been achieved so far. A study was conducted where 240 medicinal plant species were screened for antibacterial activity against an NDM-1 producing *E. coli* strain. Twelve plant extracts displayed disruption of bacterial cell wall by scanning electron microscopy. The membrane permeability caused 79.4% to 89.7% bacterial cell deaths which was observed using flowcytometry. These potential plant extracts inhibited activity of the NDM-1 enzyme *in vitro*, and IC50 value ranged between 0.50 and 1.2 ng/µl while IC50 values for ESBL is yet to be estimated. Phtyocompounds from 3 plants were isolated and their combinations were checked with antibiotics and other compounds. All the active phytocompounds showed synergistic effects when combined with colistin, meropenem, and tetracycline and reduced the MIC of the tested antibiotics. Inhibition of enzyme using plant extracts or phytocompounds seems to be a promising alternate to overcome this multi drug resistance of microorganisms hence reducing the antibiotic usage. Furthermore, structural insights of the isolated compounds against these enzymes and understanding its molecular mechanisms are required before applied clinically.

100

Bio-responsive gels containing glycoside hydrolase/gentamicin to combat *P. aeruginosa* biofilms

Nicky Thomas[1], Chelsea Thorn[1], Katharina Richter[2], Ben Boyd[3], Clive A Prestidge[1]

1. University of South Australia, Adelaide, SA, Australia

2. Basil Hetzel Institute for Translational Health Research, University of Adelaide, Adelaide, SA, Australia

3. Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia

Background: Glycoside hydrolases have emerged as potent, novel therapeutics that can disrupt biofilms, thereby increasing the susceptibility of the residing bacteria to co-administered antibiotics [1]. The broader clinical use of glycoside hydrolases such as alginate lyase (AL) is limited due to challenges in maintaining enzyme stability, adequate delivery and release of the enzyme at the site of infection. Herein, we present a nanostructured Trojan Horse carrier for AL using environment-sensing lyotropic liquid crystalline gels (LLC) [2].

Aim: To design a LLC-gel carrier based on the lipid glycerol monooleate to protect, deliver and release AL in combination with the antibiotic gentamicin (GENT) as a novel anti-biofilm strategy.

Methods: The effect of *Pseudomonas* lipase on the release of AL/GENT from LLC-gels was evaluated and the efficacy of the gel was determined over 1 week *in vitro* against biofilms formed by alginate producing *P. aeruginosa* (clinical isolate) and compared to an unformulated simple drug solution. Finally, the stability of AL after fabrication of the LLC gel was assessed.

Results: GENT and AL were released at different rates and extent from the LLC-gels (10% AL over 9 days; 60% GENT over 2 days, respectively). Addition of *Pseudomonas* lipase increased AL release >2-fold (20-30% within 2 days). The LLC-gel demonstrated similar anti-biofilm activity (2.5 log reduction in CFU) compared to unformulated solution, confirming preservation of AL activity in the LLC-gels. Interestingly the antimicrobial effect could not be sustained over extended period (>2 days) which was attributed to a gradual loss of AL activity from prolonged exposure to 37^{o}C during the assay, rather than short exposure to higher temperatures (60^{o}C) during LLC-gel fabrication.

Summary: LLC gels present a promising Trojan Horse strategy to conceal and protect biologicals such as glycoside hydrolase. The ability of infection-triggered release provides potential as a future site-specific anti-biofilm therapeutic system.

1. Kaplan, J.B., Therapeutic potential of biofilm-dispersing enzymes. International Journal of Artifical Organs, 2009. 32(9): p. 545-554.
2. Bisset, N.B., B.J. Boyd, and Y.-D. Dong, Tailoring liquid crystalline lipid nanomaterials for controlled release of macromolecules. International Journal of Pharmaceutics, 2015. 495(1): p. 241-248.

101

Not available at time of printing

Paul Bartley[1]

1. Wesley Private Hospital, Auchenflower, QLD, Australia

Not available at time of printing

102

Not available at time or printing

Rachel Thomson[1]

1. Greenslopes Private Hospital, PADDINGTON, QLD, Australia

Not available at time or printing

103

Not available at time of printing

Louise Roddam[1]

1. *University Of Tasmania, HOBART, TAS, Australia*

Not available at time of printing

104

Integrating genomics and transcriptomics to understand *Burkholderia pseudomallei* evolution in the cystic fibrosis lung

Erin P. Price[1, 2], Linda T. Viberg[2], Timothy J. Kidd[3, 4], Scott C. Bell[5, 6], Bart J. Currie[2, 7], Derek S. Sarovich[1, 2]

1. Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Sippy Downs, QLD, Australia

2. Global and Tropical Health Division, Menzies School of Health Research, Tiwi, NT, Australia

3. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

4. Faculty of Medicine, The University of Queensland, Herston, QLD, Australia

5. Lung Bacteria Laboratory, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

6. Department of Thoracic Medicine, The Prince Charles Hospital, Chermside, QLD, Australia

7. Department of Infectious Diseases and Northern Territory Medical Program, Royal Darwin Hospital, Tiwi, NT, Australia

The melioidosis bacterium, *Burkholderia pseudomallei*, is increasingly being recognised as a pathogen in patients with cystic fibrosis (CF), with approximately 30 cases documented to date. We first catalogued genome-wide variation of paired, isogenic *B. pseudomallei* isolates from seven Australasian CF cases, which were collected between four and 55 months apart. Strains showed evolutionary patterns similar to those of other chronic infections, including emergence of antibiotic resistance, genome reduction, and deleterious mutations in genes involved in virulence, metabolism, environmental survival, and cell wall components. We identified the first reported *B. pseudomallei* hypermutator in patient CF9, which was caused by a defective DNA mismatch repair protein, MutS. Further, we identified both known and novel molecular mechanisms conferring resistance to five clinically important antibiotics for melioidosis treatment. We subsequently extended this investigation by documenting the transcriptomic changes in *B. pseudomallei* in five cases. Following growth in an artificial CF sputum medium, four of the five paired isolates exhibited significant differential gene expression (DE) that affected between 32 and 792 genes. The greatest number of DE events was observed between patient CF9 strains, consistent with the hypermutator status of the latter strain. Two patient isolates harboured duplications that concomitantly increased expression of the β-lactamase gene *penA*, and a 35kb deletion in another abolished expression of 29 genes. Convergent DE in the chronically-adapted isolates identified two significantly downregulated and 17 significantly upregulated loci, including the antibiotic resistance-nodulation-division (RND) efflux pump BpeEF-OprC, the quorum-sensing *hhqABCDE* operon, and a cyanide- and pyocyanin- insensitive cytochrome *bd* quinol oxidase. These convergent pathoadaptations increase the expression of pathways that may suppress competing bacterial and fungal pathogens and that enhance survival in oxygen-restricted environments, the latter of which renders conventional antibiotics less effective *in vivo*. Treating chronically-adapted *B. pseudomallei* infections with antibiotics designed to target anaerobic infections, such as the nitroimidazole class of antibiotics, may significantly improve pathogen eradication attempts by exploiting this Achilles heel. Taken together, our work highlights the exquisite adaptability of microorganisms to long-term persistence in their environment and the ongoing challenges of antibiotic treatment in eradicating pathogens in the CF lung.

105

Induced repeat expansion: Characterising a novel mechanism for carbapenem resistance in fatal respiratory diphtheria

Brian M Forde[2, 1, 3], Andrew Henderson[4], Geoffrey Playford[4], David Looke[4], David L Paterson[1, 5], Scott A Beatson[2, 1, 3]

1. Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, QLD, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

3. Australian Centre for Ecogenomics, University of Queensland, Brisbane, QLD, Australia

4. Princess Alexandria Hospital, Brisbane, QLD, Australia

5. The Univesity of Queensland Clinical Research Centre, The university of Queensland, Brisbane, QLD, Australia

Diphtheria is a potentially fatal respiratory disease caused by toxigenic *Corynebacterium diphtheriae*. Diphtheria is rare in developed countries but remains endemic in parts of Asia, the South Pacific, the Middle East, Eastern Europe and the Caribbean. A diphtheria toxoid vaccine is available and infection is also readily treatable with penicillin or erythromycin. Although resistance to erythromycin has been recognised, Penicillin and β -lactam resistance in toxigenic diphtheria has not yet been described. Here we report a case of fatal respiratory diphtheria caused by toxigenic *C. diphtheriae* resistant to penicillin and other β-lactam antibiotics. Toxigenic *C. diphtheriae* strain BQ11 was isolated from an unvaccinated adult female in April 2011 in Australia. The infection was likely acquired from a vaccinated close personal contact with asymptomatic *C. diphtheriae* carriage. Using long-read whole genome sequencing we assembled the genome sequence of *C. dipththeriae* BQ11 to determine the genetic mechanisms of resistance. We found a 6,682 bp β-lactam resistance gene cassette that is not found in the genomes of β-lactam susceptible *C. diphtheriae*. Surprisingly, this element has been mobilised into the genome of BQ11 as a novel transposon. Remarkably, we found that transposon copy number was highly dynamic. When cultured with exposure to meropenem, selective pressure drives a rapid increase in transposon copy number and a corresponding change from a carbapenem susceptible to a carbapenem resistant phenotype.This case of fatal respiratory toxigenic diphtheria caused by β-lactam resistant *C. diphtheriae* highlights the ongoing threat posed by the potential introduction of diphtheria from endemic regions to non-immune or partially immune individuals and demonstrates threat of emergent antimicrobial resistance within this species.

106

Identifying microbial factors protective against recurrent acute otitis media

Rachael Lappan[1, 2], Kara Imbrogno[1, 3], Chisha Sikazwe[4], Denise Anderson[3], Danny Mok[3], Harvey Coates[5], Shyan Vijayasekaran[5, 6], Paul Bumbak[5, 6], Christopher C Blyth[2, 4, 5, 6], Sarra E Jamieson[3], Christopher S Peacock[1, 3]

1. The Marshall Centre for Infectious Diseases Research and Training, School of Biomedical Sciences, The University of Western Australia, Perth, WA, Australia

2. Wesfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia

3. Telethon Kids Institute, The University of Western Australia, Perth, WA, Australia

4. Department of Microbiology, PathWest, Perth, WA, Australia

5. School of Medicine, The University of Western Australia, Perth, WA, Australia

6. Princess Margaret Hospital for Children, Perth, WA, Australia

Background

Recurrent acute otitis media (rAOM, recurrent ear infection) is a common childhood disease caused by bacteria known as *otopathogens,* for which current treatments have limited effectiveness. Generic probiotic therapies have shown promise, but seem to lack specificity. We hypothesised that healthy children with no history of rAOM carry protective commensal bacteria that could be translated into a specific probiotic therapy to break the cycle of re-infection. We characterised the nasopharyngeal microbiome of these children in comparison to children with rAOM to identify potentially protective bacteria. As some children with rAOM do not appear to carry any of the known otopathogens, we also hypothesised that characterisation of the middle ear microbiome could identify novel otopathogens, which may also guide the development of more effective therapies.

Methods

We recruited children undergoing ventilation tube insertion for rAOM as cases, and age- and season-matched children with no history of AOM despite exposure to major risk factors (i.e. attendance at day care) as controls. Middle ear fluids, middle ear rinses and ear canal swabs from the cases and nasopharyngeal swabs from both groups underwent 16S rRNA gene sequencing, and a subset of samples underwent metagenomic shotgun sequencing. All middle ear fluids and nasopharyngeal swabs were also tested for respiratory viruses.

Results

The nasopharyngeal microbiomes of cases and controls were distinct. We observed a significantly higher abundance of *Corynebacterium* and *Dolosigranulum* in the nasopharynx of controls. *Alloiococcus, Staphylococcus* and *Turicella* were abundant in the middle ear and ear canal of cases, but were uncommon in the nasopharynx of both groups. *Gemella* and *Neisseria* were characteristic of the case nasopharynx, but were not prevalent in the middle ear. No additional genera were detected by metagenomic shotgun sequencing, which was taxonomically similar to the profiles produced by 16S rRNA gene sequencing.

Conclusions

Corynebacterium and *Dolosigranulum* are characteristic of a healthy nasopharyngeal microbiome. *Alloiococcus, Staphylococcus* and *Turicella* are possible novel otopathogens, though their rarity in the nasopharynx and prevalence in the ear canal means that their role as normal aural flora cannot be ruled out. *Gemella* and *Neisseria* are unlikely to be novel otopathogens as they do not appear to colonise the middle ear in children with rAOM.

Oral Abstracts

107

Harnessing Environmental Biocontrol Lactic Acid Bacteria For Food Safety and Quality

Mark S Turner[1]

1. University Of Queensland, BRISBANE, QLD, Australia

With many food poisoning outbreaks occurring and an estimated one-quarter of all food produced being wasted, food safety issues and food spoilage present major challenges both in Australia and internationally. The consumer-driven push away from chemical preservatives and towards minimal processing of foods will further strain these issues. Biocontrol strategies present one alternative preservation method which involves the use of biological-based agents such as harmless bacteria or bacteriophages to inhibit/kill undesirable microbes. In this work a large collection (n=897) of lactic acid bacteria (LAB) isolates from fresh produce (vegetables and fruits) was obtained and screened for growth inhibitory activity against two foodborne pathogens (*Salmonella*, *Listeria monocytogenes*) and a food spoilage mould (*Penicillium commune*) to identify potential biocontrol isolates. Several inhibitory isolates were obtained and demonstrated to inhibit the pathogens in cut lettuce or mould in cheese. These LAB were identified as members of the 'generally recognized as safe' *Leuconostoc*, *Lactococcus*, *Carnobacterium*, *Weissella* and *Lactobacillus* genera commonly consumed in high numbers from various fermented foods. These isolates could be applied as protective additives directly on fresh vegetables and fruits post-harvest or in cheese fermentations to improve food safety and quality.

108

Survival of *Campylobacter* through poultry processing

Lesley Duffy

Poultry products are frequently identified as a source of campylobacteriosis in Australia and across the European Union. The slaughter and processing of poultry contains a number of stages that can alter the numbers of *Campylobacter*. The processing stages of scalding, where poultry carcases are heated to temperatures between 53 °C - 58 °C for 2 – 3 min, and immersion chilling in chlorinated water, are key steps that may lower the numbers and/or prevalence of *Campylobacter* on chicken carcasses. The Weibull model is a useful tool for comparing the effectiveness of processing parameters on the inactivation of *Campylobacter*. A total of 32 isolates/strains were heated at 53 and 57 °C for 5 min and subjected to 1.10 ppm chlorine (pH 6.50) for 20 min. At lower scalding temperatures *C. coli* was found to be more heat resistant than *C. jejuni*, but not at 57 °C. Overall the *Campylobacter* isolates/strains used in this study demonstrate no unusual heat resistance although the development of a heat resistant sub-population at 57 °C should be further investigated. The isolates/strains used demonstrated a range of survival when subjected to chlorine. It is important for the poultry industry to understand the variation that may exist in *Campylobacter* strains within their flocks when subjected to the normal range of processing practices of scalding and immersion chilling in order to refine processes such that they effectively reduce viable *Campylobacter*.

109

Salmonellosis and eggs: closing the feedback loop from research to regulation

Thilini P Keerthiratne[1], Kirstin Ross[1], Howard Fallowfield[1], Harriet Whiley[1]

1. Flinders University, Adelaide, SA, Australia

Salmonellosis is a foodborne illness of public health significance. In Australia the incidence of salmonellosis has been significantly increasing over the last decade with eggs and raw egg products identified as the main source of outbreaks. Worldwide, the primary cause of salmonellosis is *Salmonella enterica* serovar Enteritidis; however, in Australia it is S. *enterica* serovar Typhimurium. Currently, in Australia it is not possible to produce eggs guaranteed to be free for *Salmonella* contamination and as such, post collection control methods are essential for protecting public health. Our research focuses on investigating the science underpinning food safety regulations for handling eggs. This research includes:

- An investigation into the impact of egg storage temperature on *S.* Typhimurium contamination. This study found that eggs stored at 4^{o}C had reduced *S.* Typhimurium penetration through the egg shell and reduced internal contamination. This evidence suggests that the Australian guidelines should include regulation to enforce refrigerated storage of eggs.

- Examination of the Australian guidelines for producing raw egg mayonnaise free from *Salmonella* contamination found that they may not be supported by evidence. Current guidelines state that the mayonnaise should be prepared daily in small batches and refrigerated immediately. However, this is study found that temperatures protected *S.* Typhimurium from low pH reducing the effectiveness of this control measure.

- Characterisation of *sous-vide* pasteurisation of eggs as an on-site method to provide eggs free from contamination. The optimum conditions for the control of S. Typhimurium and the influence on eggs properties, including viscosity, colour, thermal coagulation and the egg protein quality were identified. The acceptance and usability of the produced pasteurised eggs for the production of raw egg mayonnaise was also tested through a blind control study of chefs.

Future research examining current guidelines and food handling practices is needed to continue to inform improved regulations for better public health protection.

Oral Abstracts

110

Metagenomic profile of the bacterial community structure on poultry carcasses throughout a factory processing line

Stanley H Chen[1, 2], Narelle Fegan[3], Chawalit Kocharunchitt[2], John P Bowman[2], Lesley L Duffy[1]

1. Agriculture & Food, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Coopers Plains, Queensland, Australia

2. Tasmanian Institute of Agriculture/School of Land and Food, University of Tasmania, Hobart, Tasmania, Australia

3. Agriculture & Food, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Werribee, Victoria, Australia

Food safety issues in poultry have been gaining attention due to increasing poultry sales particularly in relation to food-borne pathogen contamination. This study aimed to examine the bacterial community structure at specific poultry processing steps, using next generation sequencing and cultural techniques. A single flock was sampled from pre-/post-scalding, pre-/post-immersion chilling and post-air chilling (10 birds per stage) as well as 10 caeca after evisceration. Total Viable Count (TVC), enumeration of *Escherichia coli* and *Campylobacter*, and determination of *Salmonella* prevalence was performed. The bacterial community structures were investigated by targeting the V4 region of bacterial 16S rRNA genes. Reductions of >4 $\log_{10}$ CFU/ml in TVC and *E. coli*, and >5 $\log_{10}$ CFU/ml in *Campylobacter* were recorded when comparing cultural counts of pre-scalding to post-air chilling. *Salmonella* was not detected in post-air chilling samples compared to 50% *Salmonella* prevalence in pre-scalding samples. The bacterial community data revealed the four most abundant bacterial phyla in all processing stages were *Bacteroidetes*, *Firmicutes*, *Proteobacteria* and *Actinobacteria*, which all together represented at least 97.1% of the bacterial population at each processing stage. At genera level, unclassified *Lachnospiraceae*, *Lactobacillus* and *Staphylococcus* were persistent bacteria in Top 10 OTUs across all sampled stages. The relative abundance of *Campylobacter* was consistently around 0.1% throughout processing with highest abundance at 0.5% in post-immersion chilling samples, whereas the cultural counts suggested the reduction of *Campylobacter* through processing. However changes of the relative abundance of *E. coli* was in agreement with its cultural counts, which decreased through processing with an increase of 7.5% between post-scalding and pre-immersion chilling. Interestingly *Faecalibacterium* and unclassified *Ruminococcaceae* became Top 10 OTUs after scalding and immersion chilling, suggesting their resistance to processing interventions. *Pseudomonas* were the most dominant bacteria (20.3%) in post-air chilling samples, indicating its role in the downstream spoilage community. The investigation offers an understanding of the bacterial community structure on chicken carcasses, how it changed, and where the contamination may occur through processing.

111

The need for speed: Development of a rapid *Campylobacter* detection system for the Australian chicken industry

Michael Mason[1], Pat Blackall[2], Jimmy Botella[1], Jillian Templeton[3]

1. SAFS, The University of Queensland, BRISBANE, QLD, Australia

2. QAAFI, The University of Queensland, BRISBANE, QLD, Australia

3. AgriScience Queensland, DAF Queensland, BRISBANE, QLD, Australia

Campylobacter is responsible for the majority of food-borne infections in Australia with poultry meat being a major, but not the sole source. The chicken industry can further enhance the quality and safety of chicken meat products by having access to faster *Campylobacter* detection methods so that real-time risk management strategies can be implemented in the processing plant. Current culture-based methods for detection and determination of levels of *Campylobacter* take two to three days; we aimed to reduce this time to two to three hours. We have taken up this challenge and have now developed a low-cost and rapid (~2 hours) detection system that has only six simple steps and requires very little hands-on time to perform. The assay is capable of distinguishing between chicken rinsate samples that are either within or exceed the industry set benchmark of 6000 *Campylobacter* cfu per carcass with a very low error rate. To achieve this, we have developed and incorporated a number of technologies that were designed to reduce both the amount of time and sample processing required. These technologies include a rapid 30 second DNA extraction technique and a rapid and equipment-free DNA amplification readout method that allows the user to visualise the results of the assay within seconds. This work was funded by the Chicken Meat Committee of the AgriFutures Australia and DAF.

112

Tracking a naturally recombinant *Campylobacter* by WGS

Jillian Templeton[1], Jan-Maree Hewitson[1], Amy Jennison[2], Pat Blackall[3]

1. AgriScience Queensland, DAF Queensland, BRISBANE, QLD, Australia

2. Forensic and Scientific Services, Health Support Queensland, Deparment of Health, Queensland, BRISBANE, QLD, Australia

3. QAAFI, The University of Queensland, BRISBANE, QLD, Australia

Campylobacter jejuni and *Campylobacter coli* remain a key cause of human gastrointestinal disease in Australia. Poultry meat is a common, although not a sole, source of these pathogens. In an industry funded project, examining the distribution of genotypes of *C. jejuni* across Australia over a three year period in chicken caeca collected at the slaughterhouse, we encountered isolates that were initially confusing in terms of species identification. The isolates were both positive in the *mapA* PCR regarded as specific for *C. jejuni* and positive in the *cueE* PCR regarded as specific for *C. coli*. In Year 1, we examined chickens in Queensland, NSW, Tasmania, WA and SA, all from one major national poultry company. The unique double-positive isolates were present only in NSW chickens. In Year 2, we examined chickens sourced from four different companies with the source farms densely populating a very small area outside Sydney. All companies (including the national company from year 1) yielded the double positive isolates. In year 3, we examined chickens from three companies with farms in a relatively dense poultry production region outside Brisbane. Only chickens from the national company examined in Year 1 were positive for the double-positive isolates. Whole genome sequencing and bioinformatics analysis of two representative double-positive isolates has been performed. The analysis showed that the two isolates were a novel ST. Core genome MLST analysis grouped the two isolates

Oral Abstracts

in with *C. coli* and found only a four gene difference in the two isolates. The results suggest this clone represents a *C. coli* strain that has acquired the *mapA* gene of *C. jejuni*. As well, the temporal and geographical distribution suggests that the strain has moved from NSW to Queensland in recent times. Further, in the more densely poultry production system outside Sydney, local horizontal transmission between farms of different companies has occurred. Local transmission was not detected in Queensland, perhaps due to the lower density of poultry farms.

113

The complement alternative pathway: another contributor to dengue virus-induced vascular leak.

Jillian Carr[1], Sheila Cabezas[1], David L Gordon[1]

1. Flinders University, BEDFORD, SA, Australia

The complement system is an important part of the body's innate response to pathogens but needs to be tightly regulated to prevent host tissue damage. The severity of dengue disease has been linked to overactivity of the complement system, in particular the constitutively active alternative pathway (AP). A major negative regulator of AP activity is factor H (FH) while factor B (FB) promotes AP activity.

Through *in vitro* studies in primary cells we have shown that DENV infection induces AP activity, particularly at the endothelial cell (EC) surface and with properties that are likely to lead to complement-mediated EC permeability: a hallmark of severe dengue disease[1]. We have further investigated the AP *in vivo*, through analysis of patient samples and in the AG129-dengue virus (DENV) mouse model, with the latter a difficult model to interpret due to the interferon (IFN)-dependency of FH and FB production during DENV infection.

Overall, it is clear that the complement AP responds to DENV infection in a manner predicted to be deleterious to the endothelium and adding another layer of complexity to the pathogenesis of dengue vascular leak syndrome. Deciphering how we can address this for therapeutic benefit is of future interest.

1. Cabezas, S., Bracho, G., Aloia, A. L., Adamson, P. J., Bonder, C. S., Smith, J. R., Gordon, D. L. and Carr, J. M. (2018) Dengue virus induces increased activity of the complement alternative pathway in infected cells. Journal of Virology (accepted May 2018).

114

Defining host restriction factors that modulate respiratory virus entry and exit from infected cells.

Patrick Reading[1, 2], Sarah Londrigan[2], Andrew Brooks[2], Fernando Villalón-Letelier[2]

1. WHO Collaborating Centre for Reference and Research on Influenza, at the Peter Doherty Instutite for Infection and Immunity, Melbourne, VIC, Australia

2. Microbiology and Immunology, The University of Melbourne, at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

Acute respiratory tract infections are important causes of morbidity and mortality worldwide, particularly in infants and the elderly. Influenza A virus (IAV), human metapneumovirus (HMPV) and respiratory syncytial virus (RSV) are major causes of viral respiratory disease. Respiratory viruses infect airway epithelial cells, resulting in virus amplification and spread. Viruses such as IAV and RSV also infect cells of the immune system, such as airway macrophages (AMΦ), however virus replication is generally blocked in these cells.

Recent studies in our laboratory used RNA-seq to investigate differences in expression of host factors between AMΦ and airway epithelial cells, in the presence or absence of IAV infection, in an attempt to identify putative restriction factors that may block virus infection. Based on these results we have focused on particular gene families where certain members were expressed at high levels in AMΦ, but not in epithelial cells. We have used overexpression and/or knockdown approaches to screen families of membrane-associated RING-CH (MARCH) ubiquitin ligases, interferon-inducible transmembrane (IFITM)-family proteins and T-cell immunoglobulin and mucin (TIM)-domain family proteins for antiviral activity against IAV, RSV and HMPV. Preliminary data indicates that overexpression of MARCH8 (but not other MARCH-family proteins) does not alter IAV entry, but does inhibit virus release from infected cells. Moreover, MARCH8 expression also inhibited replication of other respiratory viruses such as RSV. Current studies in the laboratory aim to define the mechanisms underlying the antiviral activity of MARCH8, as well as defining the role of endogenous MARCH8 in limiting respiratory virus infection.

115

How do bats clear a viral infection?

Michelle Baker[1]

1. Health and Biosecurity Business Unit, CSIRO, Australian Animal Health Laboratory, Geelong, Victoria, Australia

Bats are host to a variety of emerging and re-emerging viruses, including paramyxoviruses (Hendra and Nipah), filoviruses (Ebola and Marburg) and coronaviruses (SARS). These viruses cause no clinical disease in bats but are highly pathogenic in other susceptible hosts, including humans. To understand the ability of bats to coexist with viruses and the kinetics of viral infection, we have been using the Australian black flying fox (Pteropus alecto), the natural reservoir for Hendra virus and a variety of other viruses as a model bat species. Renewed interest in bat immunology has highlighted a number of unique adaptations in the immune system of bats that may allow them to control viral replication without activating a highly inflammatory response. Experimental infections of bats and other susceptible animal model species have also provided information on the differences in viral dissemination and immune activation between reservoir and susceptible hosts. Bats express interferons constitutively even prior to infection, allowing them to respond more rapidly compared to other species, potentially providing the immune system with a head start against pathogens. However, experimental infections have

revealed that interferons may be less critical to the final clearance of the infection and that other factors likely play a role in the differences in viral dissemination and control of replication in bats compared to susceptible hosts.

116

HIV-1 RT mutations and small molecules inhibit the interaction between RT and eEF1A and highlight its importance in uncoating, reverse transcription and replication

Daniel J Rawle[1, 2], Dongsheng Li[1], Joakim Swedberg[3], Dinesh C Soares[4], Lu Wang[1, 2], David Harrich[1]

1. Department of Cell and Molecular Biology, QIMR Berghofer Medical Research Institute, Herston, QLD, Australia

2. School of Chemistry and Molecular Biosciences, University of Queensland, St Lucia, QLD, Australia

3. Institute for Molecular Biosciences, University of Queensland, St Lucia, QLD, Australia

4. MRC Institute of Genetics and Molecular Medicine, Western General Hospital, University of Edinburgh, Edinburgh, United Kingdom

Once HIV-1 enters a cell, the viral core is uncoated by a poorly understood mechanism and the HIV-1 genomic RNA is reverse transcribed into DNA. Host cell factors are essential for these processes, however very few reverse transcription complex (RTC) binding host cell factors have been convincingly shown to affect uncoating or reverse transcription. We previously reported that cellular eukaryotic translation elongation factor 1A (eEF1A) interacts tightly and directly with HIV-1 reverse transcriptase (RT) for more efficient reverse transcription. Here we report that the surface-exposed acidic residues in the HIV-1 RT thumb domain alpha-J helix and flanking regions are important for interaction with eEF1A. Mutation of the surface-exposed acidic thumb domain residues D250, E297, E298 and E300 to arginine residues resulted in various levels of impairment of the interaction between RT and eEF1A. The best example is the strictly conserved E300 residue, where mutation significantly impaired the interaction of RT with eEF1A and virus replication in CD4+ T cells without affecting in vitro RT catalytic activity, RT heterodimerization, or RNase H activity. The impairment of RT and eEF1A interaction by the RT mutations correlated to the efficiencies of reverse transcription, uncoating and replication. This advances the structural and mechanistic detail of the key RT-eEF1A interaction in HIV-1 infection and indicates its importance in uncoating for the first time. We have also identified small molecule inhibitors of the HIV-1 RT-eEF1A interaction through a small molecule library screen using a live-cell assay. These compounds bind HIV-1 RT and inhibit infection of WT and non-nucleoside reverse-transcriptase inhibitor (NNRTI)-resistant HIV-1 mutant strains. These small molecules do not inhibit in vitro RT enzymatic activity, but disrupt the RT-eEF1A complex. Therefore these compounds have a novel mechanism of action, and using medicinal chemistry, pharmacophore modelling and molecular docking studies to improve the therapeutic effectiveness of these compounds may result in a new class of anti-HIV-1 drugs to treat NNRTI-resistant strains in infected people.

117

Identification and characteristics of the newly emerged subclades of enterovirus D68 associated with severe respiratory/central nervous system infections in Hong Kong

Pyrear SH Zhao[1], Cyril CY Yip[1], Siddharth Sridhar[1], Wang-Ngai Chow[1], Kelvin KW To[1], Kwok-Hung Chan[1], Jasper FW Chan[1], Vincent CC Cheng[1], Kwok-Yung Yuen[1], Patrick CY Woo[1], Susanna KP Lau[1]

1. The University of Hong Kong, Hong Kong, POKFULAM

Enterovirus D68 (EV-D68) caused a large outbreak in the United States in 2014. But the circulating features of EV-D68 were largely unknown. To better understand the disease impact and evolutionary dynamics of EV-D68, we studied the clinical characteristics of patients and analysed the genetic characteristics of VP1, 2C, 3D genes and the genomes. Total 10,400 nasopharyngeal aspirates (NPAs) from hospitalized patients (2010-2017) were subjected to RT-PCR to detect EV-D68. VP1, 2C and 3D genes of the positive samples were sequenced and subjected to phylogenetic analyses. Recombination Detection Program (RDP4) and Simplot version 3.5.1 were employed to predict potential recombination events. EV-D68 was detected in 40 (0.38%) NPAs from 31 children and 9 adults/elderlies. Phylogenetic analysis identified two strains of EV-D68 subclade A1, 12 of subclade B1, 12 of subclade B3 (including a 10-year-old boy presented with severe central nervous system syndromes, cardiac arrest and finally death), and 14 of clade D (including all HK strains from adult/elderly patients). Seventeen patients had pneumonia, including 6 elderly patients. A deletion of 12 nucleotides in the 5' untranslated region and distinct patterns in BC and DE loops were found in strains in clade B. We reported the first fatal case in Hong Kong caused by the newly emerged subclade B3 of EV-D68. All HK strains from adult/elderly patients were belonged to the clade D, indicating an emerging trend in the elderly population. The subclade B3 was the cause of outbreaks in Sweden and USA (2016), and a fatal case in Italy (2017) recently, indicating its potential trend to prevail worldwide. The changes of amino acid residues in the BC and DE loops of the subclade B3 might lead to changes of antigenic episodes and tissue tropism, even cause more severe diseases.

1. Yip CCY, Lo JYC, Sridhar S, Lung DC, Luk S, Chan KH, Chan JFW, Cheng VCC, Woo PCY, Yuen KY, Lau SKP. First Report of a Fatal Case Associated with EV-D68 Infection in Hong Kong and Emergence of an Interclade Recombinant in China Revealed by Genome Analysis. Int J Mol Sci. 2017 May 16;18(5).
2. Lau SK, Yip CC, Zhao PS, Chow WN, To KK, Wu AK, Yuen KY, Woo PC. Enterovirus D68 Infections Associated with Severe Respiratory Illness in Elderly Patients and Emergence of a Novel Clade in Hong Kong. Sci Rep. 2016 Apr 28;6:25147. doi: 10.1038/srep25147.
3. Wang G, Zhuge J, Huang W, Nolan SM, Gilrane VL, Yin C, Dimitrova N, Fallon JT. Enterovirus D68 Subclade B3 Strain Circulating and Causing an Outbreak in the United States in 2016. Sci Rep. 2017 Apr 28;7(1):1242. doi: 10.1038/s41598-017-01349-4.
4. Dyrdak R, Grabbe M, Hammas B, Ekwall J, Hansson KE, Luthander J, Naucler P, Reinius H, Rotzén-Östlund M, Albert J. Outbreak of enterovirus D68 of the new B3 lineage in Stockholm, Sweden, August to September 2016. Euro Surveill. 2016 Nov 17;21(46). pii: 30403. doi: 10.2807/1560-7917.ES.2016.21.46.30403. Erratum in: Euro Surveill. 2016 Nov 24;21(47):.
5. Giombini E, Rueca M, Barberi W, Iori AP, Castilletti C, Scognamiglio P, Vairo F, Ippolito G, Capobianchi MR, Valli MB. Enterovirus D68-Associated Acute Flaccid Myelitis in Immunocompromised Woman, Italy. Emerg Infect Dis. 2017 Oct;23(10):1690-1693. doi: 10.3201/eid2310.170792.

118

Ross River virus: does persisting virus and/or genome contribute to chronic disease?

Elisa X.Y. Lim[1], Penny A. Rudd[1], Lara J. Herrero[1]

1. Institute for Glycomics, Griffith University, Gold Coast, Queensland, Australia

Ross River virus (RRV) is a common mosquito-borne alphavirus that causes debilitating arthritis in thousands of Australians annually[1]. The clinical manifestations typically presented include maculopapular rash, fever, myalgia and polyarthralgia. Interestingly, 5-50% of patients experience long-term joint and muscle pain after recovery even though viraemia usually clears one week after infection[1,2]. Many mouse studies have demonstrated that RRV can replicate to high titres in bones, joints and skeletal muscle associated tissues[3]. Furthermore, RRV RNA has been detected in synovium of RRV disease patients up to five-weeks post initial clinical presentation[4]. Our goal is to determine if persisting levels of infectious virus and/or viral genome present in the joints contributes to alphavirus-induced chronic disease.

In this study, we will identify the cell types that can harbour long-term RRV viral replication and/or maintenance of viral RNA. Cells present in joint-associated tissues such as chondrocytes, macrophages, myocytes, osteoclasts and osteoblasts will be infected with RRV for up to 120 days. Viral genome transcription and/or host inflammatory responses will be measured every 1-2 weeks by gene expression analysis.

Preliminary data has shown that infectious RRV virus and viral RNA can be detected in human primary chondrocytes up to 11 and 122 days post-infection respectively. Additionally, we found changes in gene expression of several key soluble factors associated with disease pathogenesis (IL-6, MCP-1, TNF-a, etc.). Collectively, the data produced will, for the first time, demonstrate the persistence of RRV and/or its genome in cells of joints.

1. Suhrbier, A., Jaffar-Bandjee, M.-C. & Gasque, P. Arthritogenic alphaviruses—an overview. Nature Reviews Rheumatology 8, 420–429 (2012).
2. Harley, D., Sleigh, A. & Ritchie, S. Ross River Virus Transmission, Infection, and Disease: a Cross-Disciplinary Review. Clinical Microbiology Reviews 14, 909–932 (2001).
3. Morrison, T. E. et al. Characterization of Ross River Virus Tropism and Virus-Induced Inflammation in a Mouse Model of Viral Arthritis and Myositis. J. Virol. 80, 737–749 (2005).
4. Soden, M. et al. Detection of viral ribonucleic acid and histologic analysis of inflamed synovium in Ross River virus infection. Arthritis & Rheumatism 43, 365–369 (2000).

119

A Virologist in Wonderland: through the looking glass

Paul Young[1]

1. University of Queensland, Brisbane, QLD, Australia

"Curiouser and curiouser!" wrote Lewis Carroll as Alice's response to the weird and extraordinary events unfolding around her in Wonderland. I think it fair to say that this is not an uncommon experience for many scientists. At least it should be if you are working at the leading edge of discovery and amongst collegiate creativity. It has certainly been my experience – and a very rewarding and positive one.

It is an honour to have been chosen as the Rubbo Orator this year. I will take this opportunity to reflect on some of my experiences in research and academia; my journey down the rabbit hole, and to draw some lessons learned. The *Virologist in Wonderland* title is a reflection of the exciting opportunities I have had, as well as some of the unexpected turns and adventures that a career in science can often bring. Like Alice, I have met a wide range of characters (including one or two "mad hatters" – and no, I won't name them) who have influenced me along the way. Some as mentors, some as mentees and many as collaborators and friends. At this stage in my career I spend a lot of my time facilitating opportunities for my younger colleagues and I would like to share some thoughts and perspectives on a career in science.

I will also discuss some recent exciting work under way in my laboratory, on a recombinant protein engineering approach to subunit vaccine design that helps drive a more potent, protective immune response. I will outline our application of this generic recombinant platform technology to rapid response efforts to emerging disease threats as well as our progress towards a universal flu vaccine.

120

Not available at time of printing

Patrick Harris[1]

1. University of Queensland, Alderley, QLD, Australia

Not available at time of printing

Oral Abstracts

121

Refining bacterial disease surveillance: harnessing the benefits of whole genome sequencing in a public health microbiology reference laboratory

Amy V Jennison[1]

1. Public Health Microbiology, Forensic and Scientific Services, Queensland Department of Health, Brisbane, QLD, Australia

Public Health Microbiology reference laboratories fulfil a critical role in providing overarching testing and surveillance for notifiable, emerging and important pathogens. These duties require the laboratory to possess an extensive repertoire of validated assays and the ability to rapidly respond to novel threats and outbreaks. For these, among other reasons, the “one stop shop” approach of whole genome sequencing (WGS) has been embraced by microbiology reference laboratories. The ability to replace multiple labour intensive assays, with a single technique of superior typeability and discrimination, though not without its challenges, has already begun to change the workflow of reference laboratories. Some examples of implementation of real time WGS for disease surveillance in Queensland for *Salmonella*, *Shigella* and Group A *Streptococcus* will be discussed, highlighting not only the improvements made to outbreak investigations and changes to testing regimes but also where there is still further development required to complete integration of genomics into public health microbiology.

Metagenomic or deep sequencing directly on clinical specimens has more recently emerged as a valuable technique in public health microbiology. This approach may fill the gap in epidemiological data caused by the increase in culture independent diagnostic testing (CIDT) in pathology laboratories. Molecular diagnosis has many advantages for patient care, however the absence of isolates subsequently available for public health surveillance is concerning. Deep sequencing on clinical samples containing *N. gonorrhoeae*, *S. pneumoniae* and *N. meningitidis* demonstrate the utility of this technique for generating molecular epidemiological data including molecular resistance targets.

While individual public health laboratories are making in-roads with genomics, it is important that methods and reporting are not developed independently but rather with national and international standardisation in mind. Only then can WGS fully revolutionise testing strategies employed in public health microbiology.

122

Food, sex and genomics

Deborah A Williamson[1]

1. Microbiological Diagnostic Unit Public Health Laboratory, Melbourne, VICTORIA, Australia

The introduction of microbial genomics into Public Health Laboratories in Australia has been disruptive in technological terms by enabling replacement of existing phenotypic and molecular typing tests, and in organisational terms by placing complex molecular analysis at the forefront of communicable diseases prevention and control. Two of the major focus areas for translating microbial genomics into public health practice are in foodborne diseases and sexually transmitted infections. This talk will provide an overview of how genomics can be applied to the investigation, surveillance and control of these important public health problems. In particular, the emergence and control of antimicrobial resistance within these areas will be discussed.

123

Genomic evolution of *Klebsiella pneumoniae* clones: the good, the bad and the ugly

Kelly L Wyres[1], Sebastián Duchêne[1], Roni Froumine[1], Ryan R Wick[1], Claire Gorrie[1], Louise M Judd[1], Adam Jenney[2], Kathryn E Holt[1]

1. Department of Biochemistry and Molecular Biology, Bio21 Institute, University of Melbourne, Parkville, VIC, Australia

2. Department of Infectious Diseases and Microbiology Unit, The Alfred Hospital, Melbourne, Victoria, Australia

Klebsiella pneumoniae (Kp) is an infamous cause of multi-drug resistant (MDR) healthcare-associated infections, for which several MDR clones are globally distributed. In the past 30 years a small number of drug-susceptible clones have also become globally distributed, causing severe community-acquired infections. These “hypervirulent” clones are distinguished by expression of the highly serum-resistant K1/K2 capsules, plus high prevalence of acquired virulence determinants. While hypervirulence and drug resistance are usually mutually exclusive in Kp, the last few years have seen increasing reports of convergent, virulent and MDR strains – a potentially disastrous combination.

To shed light on the evolutionary forces separating MDR and hypervirulence, and the drivers of convergence, we conducted a comparative genomic analysis of 28 distinct clones. We characterised antimicrobial resistance genes, virulence genes and capsule synthesis loci (K-loci). We also explored chromosomal recombination, plasmid, phage and pan-genome diversity.

As expected, there was substantial variation in the prevalence of resistance and virulence genes, and five of six hypervirulent clones were each associated with only one K-locus. Twenty of 22 remaining clones (including 8 MDR) harboured diverse K-loci, and subsequent recombination analyses indicated the K-locus was a major recombination hot spot. In contrast, chromosomal recombination was generally rare in the hypervirulent clones, which also showed reduced pan-genome diversity driven largely by restricted plasmid diversity. We hypothesised the hypervirulent clones may be limited in their ability to uptake DNA originating from other bacterial cells. We found no evidence that anti-DNA defence systems (CRISPR/Cas and restriction-modification systems) were driving these trends, but our analyses implicated physical inhibition of DNA uptake by the thick K1/K2 capsules.

We conclude that the majority of clones undergo frequent capsule exchange through homologous recombination, indicating that the capsule is subject to strong diversifying selective pressure, However, recombination is rare in the hypervirulent clones, which seem to be generally restricted in their ability to uptake DNA through physical inhibition by their thick serum resistant capsules.

Oral Abstracts

124

The real genomic landscape of *Legionella pneumophila* in Sydney, Australia

Verlaine J Timms[1], Winkie Fong[1], Peter Howard[2], Rosie Sadsad[3], Sharon Chen[2], Vitali Sintchenko[1]

1. Centre of Infectious Diseases - Public Health, Sydney, NSW, Australia

2. Centre for Infectious Diseases and Microbiology- Laboratory Services, Westmead Hospital, Sydney, NSW, Australia

3. Sydney Informatics Hub Core Research Facilities, The University of Sydney, Sydney, NSW, Australia

Legionella pneumophila is ubiquitous and sporadically infects humans causing legionnaires disease, a severe form of pneumonia. Globally, the number of reported cases of Legionnaire’s disease has risen four-fold from 2000-2014. In 2016, Sydney, Australia was the epicentre of an outbreak caused by *Legionella pneumophila* serogroup 1 (Lp1). The causal Lp1 clone was typed as sequence type (ST) 211, an ST only ever reported in Canada. This discovery raised questions about whether this clone was new or whether it had existed in the environment previously. However there is very little known about outbreak related strains in Australia with most studies concentrating on localised outbreaks or those occurring overseas. Genomic surveillance of Lp1 would contribute to our knowledge of its evolution and can increase our ability to assist public health control measures in the event of an outbreak.

Therefore whole genome sequencing (WGS) was employed to investigate historical isolates from both clinical and environmental samples with the aim of determining the major outbreak clones in Sydney and whether there are other factors involved in outbreak related strains through analysis of the pangenome.

WGS of 87 Lp1 isolates from 1994-2015 was performed and the genomes typed using the *L. pneumophila* Sequenced Based Typing Scheme. Significantly, ST211 was found in both clinical and environmental samples since 1994. A further common outbreak strain, ST37 was also found however it was not as dominant as ST211 yet interestingly, both of these STs had highly related pangenomes. This study highlights the added value of genomic surveillance of pathogens with outbreak potential such as Lp1. It has long been thought that ST1 was the dominant clone, however this study shows ST211 is predominate in NSW and highlights the genomic similarity of this clone from Australia to another major disease causing clone ST37 from the USA and Europe.

125

Whole genome analysis of clinical Group B Streptococcus isolates from Western Australia

Lucy L Furfaro[1], Barbara J Chang[2], Charlene M Kahler[2], Matthew S Payne[1]

1. Medical School, Division of Obstetrics and Gynaecology, The University of Western Australia, Perth, WA, Australia

2. School of Biomedical Sciences, The Marshall Centre for Infectious Diseases Research and Training, The University of Western Australia, Perth, WA, Australia

Streptococcus agalactiae, commonly known as Group B Streptococcus (GBS), is a major neonatal pathogen. Due to this, pregnant women are screened by risk- or culture-based methods, resulting in intrapartum antibiotic administration to those identified as positive by screening. Culture-based methods for GBS detection provide minimal data regarding the prevalent serotypes of GBS and generate no data on other potential therapeutic targets. Whole genome sequencing (WGS), however, generates detailed information about circulating strains and population dynamics. We generated WGS data on 141 antenatal vaginal and rectal GBS isolates and 10 neonatal disease GBS isolates from Western Australia using the Illumina NextSeq. Multi-locus sequence analysis revealed the presence of 28 previously described sequence types (ST) and one new ST. Phylogenetic analysis revealed general clustering based on serotype, and neonatal disease isolates to be dispersed amongst the antenatal colonising isolates. Of the clinical isolates sequenced, the most prevalent serotypes were Ia (25.5%), III (23.2%) and V (20.5%), followed by II (14.6%), Ib (6%), VI (5.3%), IV (4%) and VIII (0.7%). Examination of surface protein genes revealed no *rib* or *bca* genes present, while fibrinogen-binding protein, laminin-binding protein and serine peptidase genes were present in 99% and *alp2* and *alp3* genes in 3% of isolates. WGS allowed the identification of gene targets that are of particular importance for estimating candidate vaccine coverage. Current vaccines targeting the capsule are in development, with a trivalent (Ia, Ib and III) vaccine in clinical trials, however, as evident from the range of serotypes present and prevalence of serotype V this may only be effective for a proportion of the population. The genes encoding surface proteins for laminin- and fibrinogen-binding, and serine peptidases, were present in the majority of the GBS genomes analysed and may be promising candidate targets if expressed. Our data suggest that, for Western Australia, current GBS vaccines in development may not provide adequate protection based on the circulating serotypes observed.

126

Generalisability among respiratory microbiota studies: when does one size fit all?

Robyn Marsh[1]

1. Menzies School Of Health Research, CASURINA, NT, Australia

Microbiomic methods are increasingly used to investigate microbial communities in the upper and lower airways. The lungs are no longer considered to be sterile, but instead are understood to contain a diverse microbiota made up of bacteria derived from the upper airways. Much research is now focused on understanding how the respiratory microbiota may contribute to the pathogenesis, treatment response and persistence of upper and lower airway infections. Microbiota research is also driving the development of novel therapies with the potential to alter airway bacterial communities and promote respiratory health (e.g. prebiotics and probiotics). Understanding whether such therapies may be effective across broad populations and different disease contexts requires consideration of the generalisability of respiratory microbiota data. The aim of this presentation is to review key elements of respiratory microbiota studies that may affect generalisability, including potential differences among populations, disease contexts, specimen types and microbiomic methods.

Oral Abstracts

127

Paradoxical Antibody: The mechanisms and treatment of antibody that exacerbates *Pseudomonas aeruginosa* lung infections.

Amy Pham[1], Emma Sheehan[2], Anthony DeSoyza[3], Ian R Henderson[2], Daniel Smith[4], Scott Bell[4], Timothy J Wells[1]

1. University of Queensland Diamantina Institute, Brisbane, QLD, Australia

2. Institute of Microbiology and Infection, University of Birmingham, Birmingham, West Midlands, United Kingdom

3. Institute of Cellular Medicine, Newcastle University, Newcastle, United Kingdom

4. Adult CF centre, Prince Charles Hospital, Brisbane, QLD, Australia

Chronic *Pseudomonas aeruginosa* lung infections are found in patients suffering from bronchiectasis and cystic fibrosis (CF) and once colonisation is established, it is difficult to remove by current methods. We previously identified a subset of patients with bronchiectasis and chronic *P. aeruginosa* infection that produced specific antibody that actually protected the infecting bacterium from both serum- and cell-mediated killing. We demonstrated that these **'inhibitory antibodies'** belong to the IgG2 subclass and target the O-antigen portion of lipopolysaccharide. Crucially, patients with high titres of inhibitory antibodies had worse lung function than infected patients with normal serum killing Two critically ill patients with this IgG2 were treated with plasmapheresis in an attempt to remove the inhibitory antibody. Both patients had immediate benefit from this treatment with a significant drop in hospitalisations, antibiotic use and markers of inflammation. Both patients lost culturable *P. aeruginosa* in their sputum for up to four months after treatment. Return of the inhibitory antibody in patients coincided with bacteria in their sputum and degrading health. Finally, we investigated the prevalence of this inhibitory antibody in a cohort of CF patients with chronic *P. aeruginosa* infection. We found that over 34% of the patients had antibody that inhibited serum-mediated killing of *P. aeruginosa*. Interestingly, titre alone was not sufficient to predict serum-inhibition, with affinity of the antibodies also important. Additionally, we identified some patients with high titres of IgA specific for the O-antigen which was also found to inhibit serum mediated killing. These findings indicate that inhibitory anti-O-antigen antibody may be a significant problem in *P. aeruginosa* lung infections, and that finding ways to remove or counteract this antibody can lead to improvement in health.

128

Macrolides: friend or foe in respiratory diseases?

Keith Grimwood[1]

1. Griffith University, Gold Coast, QUEENSLAND, Australia

Macrolides, including azithromycin, possess antibacterial, anti-inflammatory, immunomodulatory and potentially anti-viral properties. The prolonged half-life of azithromycin allows convenient once-daily dosing, leading to it becoming the most commonly prescribed macrolide for acute respiratory infections. Azithromycin is also used increasingly in a broad range of chronic pulmonary disorders characterised by neutrophilic inflammation, such as cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease and poorly-controlled asthma. Nevertheless, evidence supporting macrolides in these conditions remains inadequate.

Early viral lower respiratory infections and their interactions with the respiratory microbiota have been linked to developing asthma. Three randomised controlled trials (RCTs) reported short-term azithromycin in children with acute respiratory infections reduced the risk of hospitalisation and delayed recurrent wheezing. In contrast, four RCTs of azithromycin in infants with bronchiolitis found it did not provide clinical benefits acutely or for 6-months afterwards. Similarly, in children with pneumonia, macrolides did not shorten hospital stay, even if atypical pathogens were identified.

Long-term azithromycin potentially confers benefit at every level of the vicious cycle hypothesis of chronic lower airway inflammatory diseases driven by infection. RCTs in cystic fibrosis and bronchiectasis report on average a 50% reduction in exacerbations when azithromycin is administered for 6-24 months, although whether this is sustained is unknown.

Azithromycin's favourable pharmacokinetics also results in prolonged subinhibitory concentrations at carriage sites and selection of antibiotic resistant strains. Poor adherence increases the risk of dysbiosis and macrolide-resistance. While antibiotic-resistance is usually reversible once treatment is ceased, macrolide-resistant *Staphylococcus aureus* strains persist. The impact of macrolide-resistance at an individual and community level is uncertain. Macrolide-resistance in *Haemophilus influenzae* is associated with treatment failure in otitis media, while the effect on pneumococcal bacteraemic pneumonia is inconsistent, and whether it influences the course of chronic lower airway infections is unknown, although selecting macrolide-resistant mycobacteria in these patients is concerning.

Macrolides highlight the challenges of translating *in-vitro* observations into clinical trials and practice, while trying to avoid 'therapeutic creep' and unintended 'off-target' effects.

129

Sugar & Spice – insights into the 'diet' of *Haemophilus influenzae* in the host

Ulrike Kappler[1], Jennifer Hosmer[1], Marufa Nasreen[1], Anna Henningham[2], Rabeb Dhouib[1], Horst Schirra[3], Emmanuelle Fantino[2], Peter Sly[2], Alastair McEwan[1]

1. School of Chemistry & Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

2. Child Health Research Centre, The University of Queensland, South Brisbane, Qld, Australia

3. Centre for Advanced Imaging, The University of Queensland, St Lucia, QLD, Australia

Haemophilus influenzae is a host adapted human pathogen involved in acute and chronic respiratory diseases. The severity of disease caused by the prevalent non-typeable strains (NTHi) as well as antibiotic resistance is increasing, making the identification of alternative targets for undermining NTHi fitness essential. One possible avenue to achieve this is to target genes involved in central NTHi metabolism, thus undermining energy generation. Interestingly, very little is known about the nutritional requirements of *H. influenzae* and their relevance to in-host survival, and we have investigated this using several non-typeable strains of *H. influenzae*. Phenotypic microarrays revealed that despite genetic diversity, the carbon source utilization profiles of the NTHi strains is similar. Less than 20 carbohydrates (e.g. ribose) and carboxylic acids (e.g. lactate) were highly used by all strains, and several of these matched known components of airway mucin glycans and host cell metabolic endproducts. Nucleosides and nucleotides were the only major N- and P sources utilized by NTHi strains. All strains showed high tolerance to high pH (up to at least pH 9) and osmolyte stress, being able to tolerate high concentrations of NaCl, KCl, nitrate and nitrite. An interesting observation was that the only systematic deviations from the general profile were associated with two blood isolates suggesting a possible associated with niche adaptation. Analysis of metabolites formed and consumed in single and co-cultures of NTHi with human bronchial cells (16HBE14) and air-liquid interface differentiated epithelia by 1H-NMR revealed that NTHi consumed lactate produced by the host cells as well as glucose present in the medium, with RNAseq analyses revealing that enzymes involved in lactate conversions are highly expressed during host-NTHi co-culture. NTHi nutritional profiles are thus closely adapted to its niche in the host, and appear to be highly conserved between otherwise genetically variable strains.

Oral Abstracts

130

Molecular epidemiology of the 2013-2017 pertussis epidemic in Australia

Alice(Zheng) Xu[1], Sophie Octavia[1], Laurence Don Wai Luu[1], Michael Payne[1], Verlaine Timms[2], Alfred Chin Yen Tay[3], Anthony Keil[4], Vitali Sintchenko[2], Ruiting Lan[1]

1. UNSW, Randwick, NSW, Australia

2. Centre for Infectious Diseases and Microbiology–Public Health, Institute of Clinical Pathology and Medical Research, Sydney, NSW, Australia

3. Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, NSW, Australia

4. Department of Microbiology, Path West Laboratory Medicine WA, Perth, WA, Australia

Background: Pertussis (whooping cough) is a vaccine preventable disease caused by the bacterium *Bordetella pertussis.* Despite high vaccine coverage, pertussis has re-emerged to cause epidemic level disease. Our prior studies have shown a rapid increase in the proportion of *B. pertussis* isolates not expressing PRN (PRN-negative) during the 2008-2012 epidemic. As a new pertussis epidemic occurred in 2015 and to further our understanding of pertussis epidemiology, we characterised Australian *B. pertussis* isolates from 2013-2017, and compared their molecular characteristics with isolates from the 2008-2012 epidemic.

Methods: Whole-genome sequencing was performed on 78 clinical isolates collected from 2013-2017 to detect single-nucleotide polymorphisms (SNPs) in virulence genes and to determine their SNP profiles (SP). Together with previous 27 previously sequenced Australian *B. pertussis* isolates, a total of 105 isolates were analysed to determine their phylogenetic relationships. Western immunoblotting was performed to detect the expression of pertussis toxin (PTX), PRN and Filamentous haemagglutinin (FHA) proteins.

Results: The 78 isolates were typed into two SPs: SP13 (SNP cluster I, *ptxP3*, 96.15% [75/78]) and SP18 (non-cluster I, *ptxP1*, 3.85% [3/78]). The majority (75/78, 96.15%) of the SP13 isolates had the *prn2* and *fim3A* allele. Three non-cluster I SP18 isolates genotyped as *ptxP1-fim3A*-prn1*. The frequency of *ptxP* and *fim3* alleles were higher than those observed during the last epidemic. For PRN, 89.74% (70/78) of the isolates were found to be PRN-negative. By contrast, the percentage of PRN-negative isolates increased from 5.13% in 2008 to 77.78% in 2012. One novel PRN inactivation mechanism and a novel *fim2-3* allele were also found in this study. Importantly, we detected the first FHA-negative *B. pertussis* isolate in Australia.

Conclusion: The Australian 2013-2017 pertussis epidemic was predominantly caused by PRN-negative isolates, with local and interstate expansion. Our results suggest that *B. pertussis* continues to evolve under vaccine-induced selective pressure.

131

Translating a topical treatment for chronic upper respiratory tract infections from bench to bedside

Katharina Richter[1, 2], Sarah Vreugde[1], Tom Coenye[3], Nicky Thomas[2]

1. Ear Nose and Throat Surgery, The Queen Elizabeth Hospital, University of Adelaide, Adelaide, SA, Australia

2. Adelaide Biofilm Test Facility, School of Pharmacy and Medical Sciences, University of South Australia, Adelaide, SA, Australia

3. Laboratory of Pharmaceutical Microbiology, Ghent University, Gent, Belgium

Background: Staphylococcus aureus is associated with recurring respiratory tract infections, such as chronic rhinosinusitis and cystic fibrosis. S. aureus has the capacity to form biofilms and small colony variants (SCVs), which are pathogenic subpopulations with a preferred intracellular lifestyle. S. aureus biofilms and SCVs are linked to antibiotic tolerance and resistance, and are challenging to eradicate. Despite aggressive antimicrobial therapies and surgery, infections often recur causing ongoing morbidity and significant healthcare costs.

Aim: Preclinical validation of an antibiofilm and anti-SCV treatment targeting bacterial iron metabolism

Methods: The iron-chelator deferiprone (Def) and the haem-analogue gallium-protoporphyrin (GaPP), in solution and incorporated in a surgical wound gel, were tested for antibacterial activity using multidrug-resistant S. aureus SCVs in an intracellular infection model. The antibiofilm activity was assessed in vitro in the colony biofilm model and an artificial wound model, as well as in an in vivo infection model in nematodes (Caenorhabditis elegans).

Results: While Def alone failed to show substantial antibacterial activity, GaPP and the combination of Def-GaPP demonstrated concentration- and strain-dependent antibacterial properties. Specifically, the Def-GaPP combination significantly reduced the bacterial load in an artificial wound model (1.4 log10 reduction) and increased the survival of S. aureus SCV infected nematodes (86% survival of infected, treated worms vs. 25% survival of infected, untreated worms over 3 days). When Def-GaPP were combined with ciprofloxacin (Cip) or gentamicin (Gent), the triple combinations exceeded the antibiofilm activity of the individual compounds in the colony biofilm model against Cip- and Gent-resistant strains (5.4 log10 reduction for Def-GaPP-Cip and 3.4 log10 reduction for Def-GaPP-Gent). Moreover, Def-GaPP-Gent eradicated intracellular SCVs in human bronchial epithelial cells.

Oral Abstracts

Summary: Def-GaPP showed significant activity against S. aureus biofilms and SCVs and potentiated the activity of Cip and Gent against resistant strains. Delivered in a wound healing gel, Def-GaPP progressed to a first-in-human pilot study for the treatment of chronic rhinosinusitis.

132

Single cell viral tagging

Maria Dzunkova[1], Soo Jen Low[1], Joshua Daly[1], Li Deng[2], Christian Rinke[1], Philip Hugenholtz[1]

1. Australian Centre for Ecogenomics, School of Chemistry & Molecular Biosciences, University of Queensland, St Lucia, QLD, Australia

2. Helmholtz Zentrum München-German Research Center for Environmental Health, Institute of Virology, Neuherberg 85764, Germany

Viral discovery is accelerating at an unprecedented rate due to continuing advances in culture-independent sequence-based analyses. One important facet of this discovery is identification of the hosts of these newly characterised uncultured viruses. To this end, we have developed a single cell viral tagging approach. Fluorescently labeled anonymous virions are allowed to adsorb to unlabeled anonymous bacterial host cells which are then individually sorted as virus-host pairs, followed by genome amplification and high throughput sequencing to establish the identities of both the host and attached virus(es). We show the application of this method in the human gut microbiome including cross-tagging of viruses and bacteria between subjects.

133

Evolutionary genomics of eukaryotic algae and their microbiota

Heroen Verbruggen[1]

1. University of Melbourne, University Of Melbourne, VIC, Australia

Eukaryotic algae obtained the capacity to carry out oxygenic photosynthesis through endosymbiosis with a cyanobacterium that evolved to become the chloroplasts. These organelles still have a remnant bacterial genome encoding photosystems and several other functions. In addition, eukaryotic algae live in close association with a wide diversity of bacteria whose functions and genomes are not well understood. Some recent work from my laboratory aims to fill in some of the gaps in our knowledge of the evolutionary dynamics of chloroplast genome evolution and bacterial symbiont associations, using a group of siphonous green algae as a model. In the low-light genus *Ostreobium*, we observe an evolutionary trend towards very small, densely packed chloroplast genomes with very low rates of genome rearrangement. Many lineages of bacteria are found as intracellular endosymbionts of siphonous green algae, some of which are shown to be predominantly affected by environmental factors while others are strongly determined by host relationships. Through holobiont genome sequencing, we are currently characterising the potential nature of metabolic hand-offs and the genome dynamics of these intracellular interactions.

134

Not available at time of printing

Pierre Offre[1]

1. NIOZ Royal Netherlands Institute for Sea Research and Utrecht University, Den Burg, NOORD-HOLLAND, Netherlands

Not available at time of printing

135

Parallel systems genetics: combining TnSeq and genetically diverse mice to understand TB susceptibility

Clare M Smith[1], Richard E Baker[1], Chris M Sassetti[1]

1. University of Massachusetts Medical School, Worcester, MA, United States

The complex interplay between host and pathogen determines if an individual controls infection or progresses to disease. While abundant evidence suggests that genetic diversity contributes to the variety of outcomes, the combined effect of variation in the host and pathogen remains unclear. We developed a “dual-genome” system to unravel genetic interactions between *Mycobacterium tuberculosis (Mtb)*and its mammalian host that drive outcome to infection. Host variation was modeled using a panel of ~100 mouse strains, including the Collaborative Cross (CC) and single-gene immunological knockouts. Bacterial variation was concurrently generated using saturated libraries of transposon mutants and panels of diverse *Mtb* clinical isolates.

The wide genotypic variation in the CC panel produced remarkably diverse phenotypes upon *Mtb* infection, ranging from extreme susceptibility to progressive clearance of the pathogen. Metrics of disease that are tightly linked in the typical C57BL/6 model such as bacterial burden, dissemination, weight loss and inflammation were genetically separable in the diverse mouse strains. We identified individual polymorphic host genome regions (QTLs) underlying lung and spleen bacterial load and host control of infection independently in the CC panel.

We additionally separated the clinical disease traits into intermediate phenotypes by determining the relative fitness of thousands of bacterial mutants in the mouse panels. Host QTLs underlying differential bacterial fitness modules were identified, many of which mapped to the same host region as the clinical disease metrics. Each interaction between host and pathogen locus was defined as a host-pathogen QTL (hpQTL) that controls a specific aspect of the bacterial microenvironment and collaboratively influences global susceptibility.

Overall, the strategy of using bacterial fitness profiles as reporters of the underlying host microenvironment is a sensitive and specific method for identifying disease-modifying host polymorphisms, demonstrating the power of a dual-genome systems genetics approach to understandthe fundamental drivers of susceptibility to infection.

Oral Abstracts

136

Genomic and functional insights of bacteria affiliated with *Clostridium* Cluster IV of the core human gut microbiota

Sian Pottenger[1], Marloes Dekker Nitert[2], Paraic O Cuiv[1], Mark Morrison[1]

1. The University of Queensland Diamantina Institute, Faculty of Medicine, Translational Research Institute, Woolloongabba, Brisbane, Queensland 4102, Australia

2. School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, Australia

Functional understanding of the human gut microbiota remains hampered by a lack of cultured isolates. Here, we report on two isolates recovered from the stool of a healthy pre-adolescent child that are closely related to *Flavonifractor plautii* (strain SP1) and *Pseudoflavonifractor capillosus* (strain SP2). Both species are representative members of *Clostridium* cluster IV and have been implicated with polyphenol metabolism, but are still poorly characterized from a genomic and biochemical perspective. Cultures of *F. plautii* SP1 and DSMZ 4000^T both rapidly degrade the model polyphenol quercetin, but active growth of both strains is conditional on its complete degradation. The *F. plautii* strains including SP1 show a high degree of genome synteny, size (~4.6 Mbp) and GC content (~60%). All strains possess a chalcone isomerase (*Chi*) that is involved in the taxipholin-alphitonin conversion central to quercetin degradation, and resides within a multi-gene locus that is conserved across all these strains. Interestingly, both the *F. plautii* 16S rRNA gene and the genes from the putative *chi*-containing operon are significantly more abundant in the metagenomic data for Crohn’s disease patients compared to healthy controls available on the online annotation server MetaQuery. Both strain SP2 and *Pf. capillosus* DSMZ 23940^T are unable to degrade quercetin, and their yield is slightly reduced in its presence. However, while the 16S rRNA phylogeny suggests they are closely related, the SP2 genome possesses only a small amount of synteny and is much smaller with a lower GC content (~56%) than the *P. capillosus* and other *Pseudoflavonifractor* spp. genomes. Core genome phylogeny and Average Nucleotide Identity scores show that strain SP2 instead is the first cultured isolate of three “uncultured_Clostridium/Flavonifractor” metagenome-assembled genomes. Collectively, these studies expand our understanding of the functional attributes of a numerically predominant but poorly characterized component of the core microbiome of the human gut, and their links with diet, digestive function and health.

137

Selection for non-optimal codons in secretory signal sequences is not for weaker mRNA secondary structures in *Escherichia coli*

Yaramah Zalucki[1], Michael Jennings[1]

1. Griffith University, Southport, QLD, Australia

ABSTRACT

One hypothesis for the higher frequency of non-optimal codons at the 5’-end of genes is selection for codons that result in weaker mRNA secondary structures. Most of these studies have focused on cytoplasmic genes, and have excluded periplasmic genes from their analysis. Here we re-analysed 46 unique signal sequence variants we have generated from our previously published work on the role of non-optimal codons in the signal sequences of *Escherichia coli* secreted genes.

In this data-set, we compared the signal sequence variants to the wild-type sequence in terms of mRNA secondary structure, codon usage and relative expression levels. Using tRNA adaptation index (tAI) as a measure of codon usage, our analysis revealed that codon usage changes did not result in significant changes in predicted mRNA secondary structure. Nor were secondary structure changes correlated with changes in expression levels. However, there was a significant negative correlation with changes in tAI and their relative expression levels. These results indicate that increasing the tAI value of a signal peptide (going from non-optimal to optimal codons) results in lower expression of the signal sequence variant, and that change cannot be explained by changes in overall mRNA secondary structure. These results imply there are differing selection pressures are at present at the 5’ end of secretory genes compared to non-secretory genes. In light of these differences, whole genome analysis of codon usage should differentiate between secreted and non-secreted genes in future.

138

Out of control – Ablation of the C. albicans Histone Chaperone HIR Drives Fungal Hypervirulence

Karl Kuchler[1], Sabrina Jenull, Florian Zwolanek, Michael Riedelberger, Michael Tscherner

1. Medical University Vienna, Vienna, Austria

Fungal pathogens are considered hidden killers of mankind, as invasive fungal infections claim around 1,5 million lifes per year. Thus, fungal infections constitute a medical problem of epic proportions. Candida albicans is an extraordinary example of how a commensal pathogen can sense and integrate host immune signals to allow for rapid adaptation and survival within distinct host niches. Subtle transcriptional changes can be linked to altered chromatin organization, which profoundly affects morphogenetic cell fate decisions as observed during the C. albicans yeast-to-hyphae transition. Morphogenesis is dynamic and triggered by various host signals, involving both transient transcriptional modulation and chromatin re-modeling. We have shown that the HIR histone chaperone complex subunit facilitates replication-independent histone deposition onto chromatin but also acts as a transcriptional co-regulator of morphogenesis genes. Indeed, ablation of Hir1 decreases the sensitivity to morphogenetic signals, resulting in impaired hyphal formation. HIR1-deficient cells show markedly decreased transcriptional amplitudes of both repressing and activating genes during hyphal initiation, and a deregulated expression of extracellular proteolytic activities, suggesting that Hir1 modulates the fine-tuning of transcriptional responses during filamentation. Here, we delineate the in vivo host immune response to C. albicans hir1Δ/Δ cells. Strikingly, hir1Δ/Δ cells display a dramatic hypervirulence phenotype in a mouse model of invasive candidiasis when compared to wild type cells. Hypervirulence of hir1Δ/Δ is accompanied by dramatic increases in kidney cfu burdens, indicating that hir1Δ/Δ cells show a fitness gain and increased growth rates in vivo, resulting in their impaired clearance when compared to wild type cells. Most unexpectedly, neutrophils, but not monocytes or macrophages, are no longer recruited to kidneys harboring hir1Δ/Δ cells. Neutrophils appear “blind”

towards pathogenic signals originating from hir1Δ/Δ cells, all in all allowing for their outgrowth in vivo. We propose that hir1Δ/Δ cells alter fungal immune surveillance by either selectively impairing recruitment or activation of neutrophils that are otherwise essential for pathogen clearance. Taken together, our data suggest Hir1-mediated alterations in fungal chromatin drives immune evasion, since it reduces inflammatory responses below threshold levels otherwise required for clearance.

139

Virulence-promoting non-protein kinases: Mechanism of action and exploitation as antifungal drug targets

Sophia Lev[1], Philip Thompson[2], Julianne Djordjevic[1]

1. Centre for Infectious Diseases & Microbiology , Westmead Institute for Medical Research, 176 Hawkesbury Rd, Westmead NSW 2145, Sydney, NSW, Australia

2. Medicinal Chemistry , Monash Institute of Pharmaceutical Sciences, Parkville 3052, Melbourne, Victoria, Australia

Invasive fungal disease (IFD) poses a serious threat to human health, especially in patients immunocompromised by HIV infection or blood cancer therapy, and in organ transplant recipients. IFD affects 300 million people and causes 1.6 million deaths annually. Current antifungals are toxic, sub-optimally effective or poorly absorbed and resistance is emerging. Although novel therapies are needed urgently, no new drug classes have been introduced into clinical medicine since the echinocandins in 1986. Using the genetically tractable and major fungal pathogen, *Cryptococcus neoformans,* as a model, we showed that the inositol polyphosphate kinase (IPK), Arg1, is critically linked to virulence and IFD and potentially serves as a novel antifungal drug target[1]. Arg1 is the first of a series of IPKs acting sequentially to convert IP_3 to IP_7, a key metabolite that promotes fungal stress tolerance, metabolic adaptation and dissemination[1-3]. The direct products of fungal Arg1, $IP_{4/5}$, also convey essential virulence-associated functions, including high temperature tolerance, capsule production and normal N-linked mannosylation of enzymes involved in cell wall integrity[1]. We present evidence that the products of Arg1 promote fungal virulence by altering gene expression via direct interaction with key regulatory proteins. Work is also presented providing proof-of-principle that compounds can be developed that target fungal Arg1, but not mammalian IP_3 kinases, and hence are selective for fungi.

References: **1**. Li C, Lev S, et al. **Virulence** 2017;8:1833. **2.** Lev S, et al, **mBio**. 2015;6(3), **3.** Li C, Lev S, et al **Sci. Rep**. 2016;6:23927.

140

Metabolic control of host-pathogen interactions in fungal infection

Timothy Tucey[1], Jiyoti Verma Gaur[1], Tricia Lo[1], Paul Harrison[2], Sarah Snelgrove[3], Michael Hickey[3], Traude Beilharz[1], Thomas Naderer[1], Ana Traven[1]

1. Monash University, Clayton, VIC, Australia

2. Bioinformatics Platform, Monash University, Clayton, Victoria, Australia

3. Monash Medical Center, Clayton, Victoria, Australia

To fight off pathogens immune cells trigger a large reprogramming of metabolism, with activation of glycolysis and increased glucose consumption being a key feature. For macrophages, this sort of metabolic reprogramming has been linked to their ability to produce antimicrobial cytokines and reactive oxygen species, and drive inflammation. Microbes also reprogram their metabolism during infection. However, how metabolic reprogramming of host and pathogen interact during infection, and the impact of these metabolic interactions on disease outcomes, is poorly understood. We addressed these questions using the human fungal pathogen Candida albicans, and studying the interaction with macrophages and the importance of host glucose homeostasis in animal infection.

141

Transcriptome-wide Statistical Structure in *Saccharomyces cerevisiae* Biofilm

Kumar Selvarajoo[1], Adam Chiow[2]

1. Agency for Science, Technology & Research, Proteos, BIOPOLIS, Singapore

2. Singapore Institute of Technology, Dover Drive, Singapore

Biofilm, a colony forming cooperative response of microorganisms under environmental stress, is a major concern for food safety, water safety and drug resistance. Here, we investigated transcriptome-wide expressions of the biofilm yeast *Saccharomyces cerevisiae* in wildtype, and 6 previously identified biofilm regulating overexpression strains (*DIG1*, *SAN1*, *TOS8*, *ROF1*, *SFL1*, *HEK2*). Using a number of statistical parameters, our overall data reveal a strong transcriptome-wide invariance among all genotypes. This invariance is an indicator of order parameter that keep the global structure of biofilm stable. Thus, although single mutants may show significant favorable local expression changes, the almost unperturbed global structure will result in gradual adaptive response converging to original stable biofilm states. Our results ask for a deeper understanding of transcriptome-wide (attractor) behavior for selecting global regulatory targets for successful control of biofilm formation or progression.

Oral Abstracts

142

Recent progresses in fungal DNA barcoding

Laszlo Irinyi[1], Wieland Meyer[1], Lana Pasic[1], Minh Hoang[1]

1. Molecular Mycology Research Laboratory, CIDM, Sydney Medical School-Westmead Clinical School, , Westmead Hospital, MBI, The University of Sydney, Westmead Institute for Medical Research, Sydney, NSW, Australia

Correct and fast identification of the agents of mycoses is of great importance to enable early diagnosis and targeted antifungal therapy. DNA barcoding offers an accurate, fast, cost-effective, culture independent approach for species identification. The current primary fungal barcode is the internal transcribed spacer (ITS) region and recently a secondary barcode, the translation elongation factor 1α (*TEF1α*) has been introduced. The ISHAM-ITS database was extended with the addition of secondary barcode sequences to form the new “ISHAM BARCODE DATABASE”, which currently contains 4200 ITS and 908 *TEF1α* sequences. The application of the dual DNA barcoding system increases the ability to accurate identification of all clinically important fungal pathogens.

Recently, there has been a fundamental shift away from Sanger sequencing to next generation sequencing (NGS) allowing sequence based identification of complex samples (metabarcoding). One of those new technologies is the nanopore based long reads sequencing. To assess the advantages and pitfalls of the technology in clinical diagnosis we used the MinION™, a palm-sized sequencer to sequence DNA directly from human sputum. Our results indicate that false-positive and error-prone reads currently represent a real challenge for metabarcoding studies. To overcome these issues, more accurate taxonomy assignment algorithms and reference databases are needed.

143

SWItching it up: Purification of fungal SWI/SNF complexes reveals compositional differences from their yeast counterparts

Brianna Steed[1], Brendon Monahan[2], Alex Andrianopoulos[1]

1. The University of Melbourne, Parkville, VICTORIA, Australia

2. Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia

Talaromyces marneffei is a pathogenic fungus, endemic to South-East Asia, capable of causing lethal systemic infection in immunocompromised humans. In response to temperature changes, *T. marneffei* alternates between hyphal and pathogenic yeast growth forms: a process known as dimorphic switching. As a potential avenue to design novel anti-fungal therapies, we are interested in the molecular mechanism of dimorphic switching and how it is regulated at the chromatin level.

SWI/SNF chromatin-remodelling complexes are evolutionarily conserved, multi-subunit protein complexes, which act as DNA translocases to alter nucleosome position. These complexes regulate transcription by remodelling nucleosomes in the promoter regions of genes, facilitating access to transcriptional machinery.

Tandem-Affinity Purification (TAP) coupled with Mass Spectrometry (MS) identifies the subunit compositions of the *T. marneffei* SWI/SNF complexes; SWI/SNF and RSC. These purifications reveal compositional differences between the *T. marneffei* SWI/SNF complexes and those purified from yeast, including the identification of four novel proteins conserved across filamentous fungi. Purification of SWI/SNF and RSC from the model eurotiomycete *Aspergillus nidulans* suggests these compositional differences are conserved in other filamentous fungi, and confirms the presence of three of these novel proteins in the homologous *A. nidulans* complexes.

These findings highlight similarities and differences between the compositions of fungal SWI/SNF complexes and those previously published. Going forward, we have identified clear targets for interrogation of the role of SWI/SNF complexes in dimorphic switching, which we are investigating through a combination of genetic and biochemical techniques.

144

Engineering bacteriophages to establish a phage therapy platform for biofilm control

Karen Weynberg[2, 1], MA Schembri[2], S Beatson[2], T Peat[2], C Hartley[1], P Bond[2], JJ Barr[3], TK Lu[4]

1. Commonwealth Science Industrial Research Organisation (CSIRO), Queensland, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland, Australia

3. Monash University, Clayton, Victoria, Australia

4. Research Laboratory of Electronics, MIT, Cambridge, Massachusetts, United States of America

Biofilms consist of bacterial consortia that colonise surfaces and interfaces, and present a major global challenge in biotechnology, clinical settings, water and food industries, costing millions of lives and billions of US dollars annually. Coupled with the worldwide emergence of multi-drug resistant bacteria and an escalating antibiotic crisis, effective therapeutic antimicrobial alternatives are needed to mitigate biofilms. Viruses that infect bacteria (bacteriophage or phage) can be used to infect bacterial biofilms via a process known as ‘phage therapy’. Our research utilizes synthetic biology to engineer natural phage isolates and construct synthetic phages to effectively target biofilms. Novel engineered phage-based tools, via high-throughput evolution and synthetic component construction, CRISPR-Cas technology, and microfluidics are being utilised to control biofilm development. A library of customised ‘designer’ phages will be built and available for end-users. This project will advance state-of-the-art technology in phage engineering and biofilm experimentation with a platform of synthetic biology tools and techniques.

Oral Abstracts

145

Tripartite symbioses: Bacteriophage-bacteria-epithelial interactions

Jeremy Barr[1]

1. Monash University, Clayton, VIC, Australia

The human body is colonised by a diverse collective of microorganisms, including bacteria, fungi, protozoa and viruses, with the largest microbial community found within the gut. It is well established that our gut microbial flora has co-evolved with us, forming 'symbiotic relationships' with our bodies that are largely responsible for our overall well being. These gut-microbe symbioses impart additional beneficial functions associated with nutrient metabolism, regulation of our immune system and protection against pathogens. The smallest entity of our microbiome are the bacterial viruses. Bacteriophages, or phages for short, exert significant selective pressure on their bacterial hosts, undoubtedly influencing the human microbiome and its impact on our health and well-being. Phages colonise all niches of the body, including the skin, oral cavity, lungs, gut, and urinary tract. As such our bodies are frequently and continuously expo

ed to diverse collections of phages. Despite the prevalence of phages throughout our bodies, the extent of their interactions with human cells, organs, and immune system is still largely unknown. Phages physically interact with our mucosal surfaces, are capable of bypassing and entering epithelial cell layers, disseminate throughout the body and may manipulate our immune system. Here, I will discuss our lab's research investigating the diverse ways in which phages interact and influence the human body and propose a novel concept of tripartite symbioses between phages, their bacterial hosts, and the human gut epithelium.

146

The New Kid on the Block - A Specialised Secretion System During Bacterial Sporulation

Christopher Rodrigues[1]

1. The ithree institute, University of Technology Sydney, Ultimo, NSW, Australia

Bacteria employ large protein complexes called specialised secretion systems to transport proteins across their envelope. These nanomachines are notorious in Gram-negative pathogens where they play important roles in the infection of host cells. In this instance, they transverse the inner and outer membranes and utilize ATP hydrolysis in the cytoplasm to energize secretion of proteins across the outer membrane. Interestingly, the problem of transporting proteins across two membranes is not unique to Gram-negative bacteria. In Gram-positive, spore-forming bacteria such as the model *Bacillus subtilis* and the pathogen *Clostridium difficile,* a double-membrane assembles around the developing spore: one membrane derived from the mother cell and another from the spore. A protein complex (called the A-Q complex) with remote homology to specialised secretion systems spans these two membranes and is essential for spore development. This transenvelope complex has been hypothesized to function as a channel for molecular transport between these two cells.

There are many outstanding questions surrounding the A-Q complex: Is it a secretion complex and what does it secrete? What does it look like? How is it assembled? Do we even have the complete parts list for this complex?

In this talk, I will share seminal work that has helped define the A-Q complex as a new type of specialised secretion system with a specific role during bacterial sporulation.

147

Identification and characterisation of a new family of conjugative plasmids in *Clostridium perfringens*

Thomas D Watts[1], Callum J Vidor[1], Milena M Awad[1], Dena Lyras[1], Julian I Rood[1], Vicki Adams[1]

1. Infection and Immunity Program, Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Vic, Australia

Clostridium perfringens produces an extensive arsenal of toxins that are involved in human diseases ranging from mild enterotoxaemia and food poisoning to potentially fatal clostridial myonecrosis. Many *C. perfringens* toxins are encoded by genes found on members of the pCW3-family of conjugative plasmids. These plasmids have a high level of sequence similarity, including 11 genes that constitute the unique *tcp* conjugation locus. Recently, another series of plasmids, the pCP13-like family, have been shown to harbour important toxin genes, including genes that encode the novel binary clostridial enterotoxin, BEC. This family of plasmids was thought to be non-conjugative, but recent studies have shown that an approximately 25 kb region encoded by pCP13 has similarity to the *cst*conjugation locus of the *Paeniclostridium* (formerly *Clostridium*) *sordellii* toxin plasmid pCS1-1 and a putative conjugation locus on the *Clostridium botulinum* toxin plasmid pCLL. To determine if pCP13 was conjugative, a genetically marked derivative was constructed by insertionally inactivating the *cpb2* toxin gene with an erythromycin resistance determinant. The marked pCP13 derivative transferred at high frequency to a plasmid-free recipient. One presumed product (PcpB4) of the putative pCP13 conjugation locus has a conserved type 4 secretion system (T4SS)-like VirB4 ATPase domain. To determine if the *pcpB4* gene was required for conjugation it was insertionally inactivated and the resultant mutant assessed in a conjugation assay. The results showed that it had a greatly reduced transfer frequency (seven orders of magnitude lower) compared to wild-type pCP13. The mutant was complemented *in trans* with the wild-type *pcpB4* gene, which restored the transfer frequency to wild-type levels. Cross-complementation of the *pcpB4* mutant with its *cstB4* homologue (51.9% amino acid sequence identity) from *P. sordellii* partially restored its ability to transfer. In conclusion, we have identified a novel conjugation locus that has now been shown to be functional in *C. perfringens*. This study has significant implications for our understanding of the movement of toxin genes in this important pathogen.

Oral Abstracts

148

Genetic characterisation of Salmonella Typhi and a novel flagellatropic bacteriophage during phage burst.

Rhys Dunstan[1, 2], Derek Pickard[2], Josh Hardy[3], Rhys Grinter[1], David Goulding[2], Claire Cormie[2], Fasseli Coulibaly[3], Gordon Dougan[2], Trevor Lithgow[1]

1. Infection & Immunity Program, Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton 3800, VIC, Australia

2. Wellcome Trust Sanger Institute, Hinxton, Cambridgeshire CB10 1SA, United Kingdom

3. Infection & Immunity Program, Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University, Clayton 3800 , Vic, Australia

Bacteriophages, or phages, are viruses that specifically infect bacteria. The interactions between phages and their bacterial hosts are widespread in nature and are often very complex - many of which influence the ability of a phage to replicate inside its bacterial host. Due to the increased interest in utilising phages as therapeutic tools to combat antibiotic resistant bacterial infections, it is necessary to gain a better understanding of their basic biology. However, there is limited knowledge describing the molecular mechanisms underlying their infectious cycles during phage burst and eventual death of the bacterial cell. We have isolated a novel bacteriophage, YSD1, capable of infecting a variety of different bacterial species, and have solved its structure using single-particle cryo-electron microscopy. Using a *Salmonella Typhi* host, we determined the infection kinetics of YSD1, discovering that it requires functional flagella for infection. Using next-generation RNA-sequencing approaches, analysing both phage and host, the mechanisms used by YSD1 to modulate its *S.* Typhi host just prior to phage burst were characterised. This approach also allowed us to determine when during the infection process are specific phage genes expressed, aiding in the characterisation of several genes of unknown function. This work provides genetic understanding of the interactions between a flagellatropic bacteriophage and its *Salmonella* host and has the potential to be exploited for the development of novel anti-bacterial molecules.

149

Genetic Composition and Regulatory Control of IncA/C Plasmid Conjugation

Steven J Hancock[1], Minh Duy Phan[1], Alvin Lo[1], Kate M Peters[1], Brian M Forde[1], Zhen Luo[2], Bostjan Kobe[1], Ji Yang[3], Richard A Strugnell[3], David L Paterson[4], Timothy R Walsh[5], Scott A Beatson[1], Mark A Schembri[1]

1. Australian Infectious Diseases Research Centre, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

2. University of Queensland, ST LUCIA, QLD, Australia

3. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, VIC, Australia

4. The University of Queensland Centre for Clinical Research, Brisbane, QLD, Australia

5. Department of Medical Microbiology and Infectious Disease, Cardiff University, Cardiff, United Kingdom

Plasmids are major vehicles for the carriage and spread of antibiotic resistance genes to all major classes of antibiotics. IncA/C plasmids are a significant group of broad-host range plasmids associated with the carriage of multiple resistance genes in enteric human pathogens, including resistance to extended spectrum cephalosporins and last-line carbapenems. Thus, IncA/C plasmids have contributed to the emergence of Carbapenem Resistant *Enterobacteriaceae* (CRE), an urgent threat to public health due to their extensive antibiotic resistance profile, including resistance to last line carbapenems. Despite their impact on the dissemination of antibiotic resistance genes, our current understanding of the genetics of IncA/C conjugation is limited. In this study, we utilised hyper-saturated transposon mutagenesis coupled with transposon directed insertion site sequencing (TraDIS) to determine the complement of genes required for conjugation of the prototype IncA/C plasmid pMS6198A. In total, 27 genes were identified, including all 17 predicted and known conjugation genes, two known regulatory genes (*acaDC)* and eight genes not previously associated with conjugation. The eight novel genes were individually mutated and the resulting mutant plasmids were examined for their ability to conjugate. Five of the eight novel genes were confirmed to significantly affect conjugation. One of these genes, which we refer to as *acaB*, is a novel regulator that acts at the top of the hierarchical cascade of conjugation control. Our data demonstrate that AcaB activates conjugation via upregulation of *acaDC*, a controlling element that in-turn directly activates the transcription of genes involved in the production of conjugative pili and DNA transfer. Overall, this work has led to the identification of novel genes associated with IncA/C conjugation, thus advancing our understanding of the spread of this important group of broad-host range multidrug resistance plasmids.

150

Vibrio cholerae biofilm matrix assembly and function

Fitnat Yildiz[1]

1. University of California, Santa Cruz, Santa Cruz, CA, United States

Biofilms, microbial communities encased in an extracellular matrix, are the dominant lifestyle of bacteria. Biofilm formation enhances environmental survival and transmission of the human pathogen *Vibrio cholerae*. Understanding the mechanisms involved in biofilm formation and maintenance is critical for developing strategies to control cholera epidemics. In this presentation, I will describe the components of *V. cholerae* biofilm matrix and their role in biofilm matrix assembly. I will also describe structure-function relationship of the matrix protein RbmA and Vibrio exopolysaccharide which is required for biofilm stability.

Oral Abstracts

151

Folding and Froth: Glycosylation in yeast in protein biosynthesis and sparkling wines

Lucia F Zacchi[1], Cassandra L Pegg[1], Danila Elango[1], Edward D Kerr[1], Suchada Niamsuphap[1], Benjamin L Schulz[1]

1. University of Queensland, St Lucia, QLD, Australia

Protein glycosylation is conserved in eukaryotes from yeast to humans, and is critical in protein folding and in regulating protein functions. N-linked glycosylation of nascent polypeptides takes place in the Endoplasmic Reticulum, and genetic or chemical inhibition of this process lowers the efficiency of protein folding. We developed Data Independent Acquisition SWATH Mass Spectrometry glycoproteomic methods to map site-specific glycosylation in folding and mature proteins. This showed that unglycosylated proteins are retained in the ER, and that this effect is synergistic, with lack of glycosylation at multiple sites leading to enhanced retention. When yeast glycoproteins fold correctly, they traffic through the Golgi and are secreted. To measure the effect of inefficient glycosylation on glycoprotein function, we used this secretome from yeast with defects in N-glycosylation together with a modified thermal proteome profiling approach to efficiently assay the global consequences of site-specific glycosylation defects on glycoprotein thermal stability. Finally, as the yeast secretome contained abundant and diverse glycoproteins, we used glycoproteomic mass spectrometry to investigate their presence in industrially relevant spent media - sparkling wine. This identified highly abundant and structurally heterogeneous O-glycosylated yeast proteins which are important for sensory properties and foam formation.

152

Salmonella sweet talking it's way around host innate responses

Joshua PM Newson[1], Tania Wong Fok Lung[1], Nichollas Scott[1], Cristina Giogha[2], Nat F Brown[3], Jaclyn S Pearson[2], Elizabeth L Hartland[2]

1. Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, Parkville, Victoria, Australia

2. Centre for Innate Immunity and Infectious Diseases, Hudson Institute Of Medical Research, Clayton, Victoria, Australia

3. Michael Smith Laboratories, University of British Columbia, Vancouver, British Columbia, Canada

Pathogenic serovars of Salmonella utilise two type-three secretion systems to deliver distinct cohorts of effector proteins into host cells during infection. These effector proteins interact with specific human proteins to subvert normal cellular processes, thus impairing the ability of host cells to respond to the invading bacteria. Our research focuses on the SseK family of Salmonella effectors, which to date have unknown function in Salmonella infection. This effector family shows strong sequence homology to the effector protein NleB1 from enteropathogenic E. coli. We recently showed that NleB1 is a novel N-acetylglucosamine transferase that modifies host death domain proteins with a single GlcNAc molecule and thus inhibits programmed cell death and inflammation. The SseK effectors have the same catalytic motif as NleB1, therefore, we predict that the SseKs are also glycosyltransferases. We aim to identify the host substrates of the SseKs and to characterise the post-translational modification catalysed by these effectors.

So far, our work has established that SseK1 and SseK3 indeed function as glycosyltransferases, and catalyse a rare post-translational modification in which the sugar N-acetylglucosamine is transferred to arginine residues of host proteins. Immunofluorescence analysis suggests SseK1 and its glycosylated substrate are localised throughout the host cell, whereas SseK3 and its substrate were localised to the host Golgi. Next, we developed an approach combining immunoprecipitation and mass spectrometry to identify the glycosylated host substrates of these effector proteins. SseK1 was found to glycosylate arginine residues of the host protein TRADD, which functions as an adaptor molecule in both inflammatory and cell death signalling pathways. Further, we found that SseK3 glycosylates several components of these same pathways, suggesting the function of this effector family may be to enact a blockade of multiple host signalling pathways in order to survive and replicate. Ultimately, this work suggests that Salmonella engages in a broad program of interference with host cell signalling during infection.

153

Glycan-glycan interactions between host glycans and pathogen glycans: Role in colonisation/adherence.

Christopher Day[1], Tsitsi D Mubaiwa[1], Evgeny Semchenko[1], Elizabeth N. H. Tran[2], Freda E.-C Jen[1], Johnson Mak[1], Thomas Haselhorst[1], Victoria Korolik, Kate Seib[1], Renato Morona[2], Michael Jennings[1]

1. Institute for Glycomics, Griffith University, Gold Coast, Queensland, Australia

2. Department of Molecular and Cellular Biology, University of Adelaide, Adelaide, South Australia, Australia

Cells from all domains of life express surface carbohydrates, which are typically linked to protein or lipid and are known as glycans. Cell-to-cell contact, mediated by glycan-glycan interactions is considered insignificant with research focused primarily on protein-glycan or protein-protein interactions[1-3].

It has recently been shown in four Gram-negative bacteria that the lipooligo/polysaccharide (LOS/LPS) on the bacterial surface can directly bind host glycans with high affinity and are important in the adherence to host cells. By screening the LOS/LPS of *Campylobacter jejuni*, *Shigella flexneri*, *Salmonella typhimurium*, and *Haemophilus influenzae* we identified >300 different glycan-glycan interactions (by glycan array) and verified 66 pairs using surface plasmon resonance (SPR) and isothermal calorimetry (ITC). The highest affinity interaction identified in this study was between human blood group B antigen and the molecular mimic of asialo GM1 produced by *C. jejuni* with a dissociation constant (K_D) of ~100nM (140nM by SPR; 98nM by ITC)[4].

We have now screened a range of polysaccharides of several other pathogens including Neisseria spp. and *Pseudomonas aeruginosa* using glycan array, identifying a further ~200 novel glycan-glycan interactions. This screen has identified the highest affinity glycan-glycan interaction observed so far, with the K_D between the LPS of *N. meningitidis* and Thomsen–Friedenreich antigen of 13nM[5].

To date only very limited structural information about glycan-glycan interactions is available. Using a wide range of multidisciplinary techniques including molecular modelling and nuclear magnetic resonance, we attempt to elucidate glycan-glycan structures at an atomic level.

Oral Abstracts

We have shown that high affinity glycan-glycan interactions between bacterial pathogens and the host are wide-spread. The highest affinity interactions are between bacterial host mimicking structures and host glycans. Here we also propose a role for glycan-glycan interactions in viral pathogens. Glycan-glycan interactions are a new paradigm in interactions between these ubiquitous biomolecules in biological systems.

1. 1. Moran, A. P., Gupta, A. & Joshi, L. Sweet-talk: role of host glycosylation in bacterial pathogenesis of the gastrointestinal tract. Gut 60, 1412-1425 (2011).
2. 2. Spillmann, D. & Burger, M. M. Carbohydrate-carbohydrate interactions in adhesion. J Cell Biochem 61, 562-568, (1996).
3. 3. Varki, A. Selectin ligands. Proc Natl Acad Sci U S A 91, 7390-7397 (1994).
4. 4. Day, C. J. et al. Glycan:glycan interactions: High affinity biomolecular interactions that can mediate binding of pathogenic bacteria to host cells. Proc Natl Acad Sci U S A 112, E7266-7275, (2015).
5. 5. Mubaiwa, T. D. et al. The glycointeractome of serogroup B Neisseria meningitidis strain MC58. Sci Rep-Uk 7, (2017).

154

Utilisation of the sugar raffinose dictates disease progression in *Streptococcus pneumoniae*

Vikrant Minhas[1], Claudia Trappetti[1], Richard Harvey[1], Lauren McAllister[1], James Paton[1]

1. School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia

Streptococcus pneumoniae (the pneumococcus) is a leading cause of human mortality and morbidity, yet is more commonly carried asymptomatically in the human nasopharynx. However, the mechanisms underlying pneumococcal transition from commensal to pathogen are poorly understood, largely due to its vast genetic diversity. *S. pneumoniae* is subdivided into >90 serotypes based on the capsular polysaccharide they produce, which is superimposed on >12000 clonal types distinguished by multi-locus sequence typing. As current pneumococcal vaccines only protect against a maximum of 23 serotypes, and *S. pneumoniae* develops antibiotic resistance rapidly, new drug and vaccine targets are urgently needed.

Previous studies have shown that even closely related pneumococcal strains within the same serotype and sequence type (ST) can display variations in virulence, related to their isolation site in humans. Serotype 14 (ST15) and 3 (ST180) clinical isolates derived from the blood exhibited a tendency to cause septicaemia and/or pneumonia, while ear isolates caused otitis media and meningitis. Genomic comparisons performed between a blood and ear isolate, each from both ST15 and ST180, identified single nucleotide polymorphisms (SNPs) in the raffinose uptake genes *rafR* and *rafK,* respectively. Growth and qRT-PCR assays with raffinose as the sole carbon source showed that the blood isolates utilised raffinose more efficiently than the ear isolates, whereas there was no difference in ability to utilise glucose. Swapping *rafR* alleles between the ST15 blood and ear isolates led to a switch in the above *in vitro* characteristics. Strikingly, the *rafR* swapped strains also showed a simultaneous swap in the disease profiles between the blood and ear isolates. Now the *rafR* swapped blood isolate caused otitis media and meningitis significantly more than the *rafR* swapped ear isolate, which instead displayed an increased capacity to cause pneumonia. These results suggest that variations in the *rafR* sequence that affect the ability to utilise raffinose play a significant role in dictating tissue tropism and pneumococcal disease progression.

155

High frequency changes in pilin glycosylation patterns of epidemic serogroup A *Neisseria meningitidis* strains in the African meningitis belt

Freda E.-C. Jen[1], Ben L. Schulz[2], Gerd Pluschke[3], Michael M. Jennings[1]

1. Institute for Glycomics, Griffith University, Gold Coast, QLD, Australia

2. School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD, Australia

3. Swiss Tropical and Public Health Institute, Basel, Switzerland

Neisseria meningitidis is an important human pathogen that can cause rapidly progressing life threatening meningitis and sepsis in humans. The key processes that mediate the transition from harmless carriage of the bacterium in the nasopharynx (the case for the vast majority of colonised hosts) to invasive disease are largely undefined. Pili of pathogenic Neisseria are major virulence factors associated with adhesion, twitching motility, auto-aggregation, and DNA transformation. Pili of *N. meningitidis* are subject to several different post-translational modifications including glycosylation. Genes involved in pili glycosylation are phase-variable (high frequency, reversible on/off switching of expression). This pili-linked glycan is required for pili to optimally engage human platelet-activating factor receptor to mediate adherence to human epithelial cells.

In the meningitis belt of sub-Saharan Africa, meningococcal epidemics occur in cycles and are associated with clonal waves of *N. meningitidis* carriage and invasive disease. In the framework of longitudinal colonization and disease studies in Ghana and Burkina Faso, meningococcal isolates belonging to the closely related hypervirulent A:ST-5, A:ST-7 and A:ST-2859 clones have been collected from 1998-2011 during meningococcal outbreaks. A comparative whole genome sequencing study with 100 of these isolates identified the pilin glycosylation (*pgl*) locus as one hot spot of recombination. Frequent exchange of *pgl* genes in *N. meningitidis* by lateral gene transfer results in differences in the glycosylation patterns of pilin and of other major cell surface protein antigens. In this study, we looked at phase variation of the *pgl* genes of representative strains. The glycan structures resulting from different *pgl* alleles were determined by mass spectrometry. Our results indicate that the basal sugar is masked by various mono- or di-saccharide structures which are variable due to the phase variable expression of *pgl* genes. This is a strong indication for bacterial adaptation to evade host immunity.

156

Not available at time of printing

Gemma Robertson

Not available at time of printing

157

Q fever in North Queensland - The changing epidemiology

Robert Norton[1]

1. Townsville Hospital, Douglas, QLD, Australia

Reported cases of Q fever in Australia have been largely confined to workers in the abbatoir and cattle industries. The introduction of the National Q fever Management program in 2001 has seen a significant fall in cases of Q fever both nationally and in Queensland (3.5/100,000). The vaccine is largely offered to workers in the cattle and meat industries. North Queensland however, continues to have one of the highest rates of Q fever in the country with clustering of cases ranging from 6.7 - 24.9 per 100,000 population. A serum survey of 1522 blood donations collected by the Australian Red Cross Blood Service in North Queensland, showed that 3.5 % of all serum samples tested from asymptomatic donors, were positive for antibodies to phase II *Coxiella burnetii* antigen. It has been shown that in this region now, up to 60% of confirmed cases of Q fever do not have any occupational exposure to cattle. Animal seroprevalence studies have shown that 16.8% of beef cattle are seropositive. This was less than the seropositivity of macropods (30.4%), bandicoots (23.9%), but more than possums (10.7%). Most cases are seen in the months immediately after the wet season. Geographic mapping of cases in the region showed clustering of cases around newer suburbs bordering on bushland. At risk occupational groups have also changed with an apparent increase in landscapers acquiring the disease. We hypothesise that an increase in native wildlife numbers seen immediately following the wet season together with an increase in human habitation on semi rural acreage, is the reason for the increase of human acute Q fever cases in the region.

158

Can we develop of a diagnostic assay to detect and monitor rheumatic heart disease?

Brenda Govan[1], **Georgina Price**[1], **Catherine Rush**[1], **Robert Norton**[2], **Natkunan Ketheesan**[3]

1. James Cook University, Townsville, QLD, Australia

2. Microbiology, The Townsville Hospital, Townsville, QLD, Australia

3. University of New England, Armidale, QLD, Australia

Rheumatic Heart Disease (RHD) is the chronic heart valve damage caused by episodes of acute rheumatic fever (ARF) which can occur following repeat infections with Group A Streptococcus (GAS). The burden of ARF and RHD is significant and borne exclusively by the socioeconomically disadvantaged. Its stark distribution across socio-economic lines is manifested in the glaring global disparities in prevalence between developing and developed countries and within developed countries such as Australia and New Zealand, between the indigenous and non-indigenous populations.

Despite the impact, there are significant gaps in the understanding of RF/RHD pathology. A proposed mechanism of the autoimmune activity in RF/RHD is cross-reactivity of anti-GAS M protein antibodies with host proteins such as cardiac myosin (CM) in the heart. It is hoped that increased understanding of this aspect of ARF/RHD pathogenesis could lead to the development of an auto-antibody based test to detect and potentially monitor ARF/RHD heart damage.

Using the Rat Autoimmune Valvulitis model of ARF/RHD, the aims of this study were to map GAS M protein stimulated antibodies to particular regions within the S2 sub-fragment of CM and to demonstrate an association between antibody reactivity to these S2 regions and the development of valvulitis as evidenced by echocardiographic (Echo), electrocardiographic (ECG) and histological findings.

Lewis rats were injected with either pooled peptides, recombinant GAS M5 protein or porcine CM (pCM). Enzyme-linked immunoassay found significant elevation of IgG reactive to two S2 peptides in the sera of rats injected with rM5. Examination of P-R intervals on ECG found statistically significant elongation in groups administered repeat injections of pooled peptides. Collectively, all Echo, ECG and histology findings demonstrated the development of valvulitis in the rats, concurrent with the presence of elevated serum IgG reactivity to the S2 sub-fragment. These two epitopes, along with other's identified in previous studies, warrant further examination of their sensitivity and specificity to ARF/RHD in human RF/RHD patients.

159

Exploration of the upper respiratory tract microbiota of remote Australian Aboriginal children – implications of season and household occupancy.

Kyra Cottrell[1], **Eva Grahn Håkansson**[2], **Amanda Wood**[3], **Seweryn Bialasiewicz**[4], **Diane Diane Maresco-Pennisi**[1], **Jasmyn Adams**[3], **Josephine Ferguson**[3], **Matthew Brown**[3], **Kristian Roos**[5], **Robert Ware**[6], **Anders Cervin**[1, 7], **Andrea Coleman**[1]

1. UQCCR, University of Queensland, Brisbane, QLD, Australia

2. Umeå University, Umeå, Sweden

3. Deadly Ears Program, Queensland Health, Brisbane, QLD, Australia

4. Queensland Paediatric Infectious Diseases Laboratory, Brisbane, QLD, Australia

5. ENT Department, Lundby Hospital, Gothenburg, Sweden

6. Griffith University, Brisbane, QLD, Australia

7. Royal Brisbane and Womens Hospital, Brisbane, QLD, Australia

Background

The aim of this study was to explore the upper respiratory tract (URT) microbiota of Australian Indigenous children from a remote Queensland Indigenous community and analyse the impact of season and household occupancy on microbiota composition.

Methods

Australian Indigenous children aged from 2-7 years were recruited from a remote Indigenous community in Queensland. Swabs were taken from the nose, buccal cavity and tonsils. We performed culture-based analysis with Vitek MS MALDI-TOF (bioMérieux) to identify isolates. Differences in the presence of bacterial species in relation to season and household occupancy were analysed using logistic regression (STATA/IC 15.0).

Results

A total of 59 children were recruited, mean age = 57 months (SD = 13), 26(44%) were male. The majority attended school/daycare (n=53, 90%). Range of household occupancy r=3-12, mean=5 (SD=2). Samples were collected in spring (n=29, 49%), autumn (n=23, 39%), and winter (n=7, 12%). A total of 163 bacterial species were identified. The most prevalent were *Streptococcus mitis/oralis* (n=55, 93%), *Streptococcus parasanguinis* and *Streptococcus pneumoniae* (both n=44, 75%), and *Haemophilus influenzae* (n=43, 73%). In relation to season, *Gemella haemolysans* (OR=2.7), *Haemophilus haemolyticus* (OR=7.2), *Neisseria mucosa* (OR=8.4)and *Prevotella* species (OR=6.1) were detected significantly more often in swabs collected in winter(all p<0.05), . Lactobacillus species were significantly more prevalent in swabs collected in spring (OR=0.4, p=0.04). There was no difference in respiratory pathogens *H. influenzae, S. pneumoniae, or M. catarrhalis.* We observed a nominal increase in the presence of *H. influenzae* when household occupancy was >7 (increase from 70 to 86%, p=0.46). Otherwise, no changes to the microbiota were observed in relation to household occupancy.

Conclusions

Using culture-based analysis we demonstrated a broad microbiota in the URT of Australian Indigenous children. Respiratory pathogens were highly prevalent in the cohort. Season appeared to impact the URT microbiota, although not for the main respiratory pathogens. In contrast, we saw no significant effect of household occupancy on URT microbiota.

160

Diagnosing *Chlamydia psittaci* and *Chlamydia pecorum* in less than an hour using rapid novel isothermal amplification assays

Martina Jelocnik[1], **Mominul Islam**[1], **Danielle Madden**[1], **Cheryl Jenkins**[2], **James Branley**[3], **Scott Carver**[4], **Adam Polkinghorne**[1]

1. University of Sunshine Coast, BRISBANE, QLD, Australia

2. Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle, NSW, Australia

3. Nepean Hospital, Penrith, NSW, Australia

4. University of Tasmania, Hobart, Tasmania, Australia

Chlamydia psittaci and *Chlamydia pecorum* are important veterinary pathogens, with the former also being responsible for zoonoses, and the latter adversely affecting koala populations in Australia and livestock globally. In Australia, the diagnosis and detection of these organisms is costly, laborious, not standardised and mainly restricted to research, challenging efforts to manage and treat infected hosts. The ability to provide a rapid detection of such infections becomes of increasing significance when zoonotic transmission is suspected for *C. psittaci*, and is also attractive for *Chlamydia* detection in wild animals such as koala due to the typical logistics associated with field sampling and treatment. Loop Mediated Isothermal Amplification (LAMP) assays are popular for use in pathogen diagnostics. We have developed and evaluated rapid and robust *C. psittaci*-specific and *C. pecorum*-specific LAMP assays for detection of these organisms in either laboratory or POC settings.

LAMP assays, run in a Fluorometer as well as thermal block targeted a (i) *C. psittaci*-specific Cps_0607 gene; and (ii) 209bp region of a *C. pecorum*-specific gene CpecG_0573, respectively, and were evaluated using a range of samples previously screened using species-specific qPCRs. Both LAMP assays were demonstrated to species-specific, highly reproducible and to be able to detect as little as 10 genome copy number/reaction, with a mean amplification time of 14 and 24 min for *C. psittaci* and *C. pecorum*, respectively. When testing clinical samples, the overall congruence between the newly developed LAMP assays and qPCR was 92.3% for *C. psittaci* (91.7% sensitivity and 92.9% specificity); and 84.1% for *C. pecorum* (90.6% sensitivity and 77.4% specificity). We also performed a pilot study using the *C. psittaci* LAMP assay at the Scone Equine Hospital where we demonstrated utility of the LAMP tests.

A recent Australian equine *C. psittaci* epizootic (with documented zoonotic events) and rampantly spreading chlamydiosis in koalas further demonstrates the need for POC assays to rapidly diagnose these pathogens. With further development and a focus on the preparation of these assays at the POC, it is anticipated that both tests may fill an important niche in the repertoire of ancillary diagnostic tools available to clinicians.

161

The role of nicotinamide adenine dinucleotide (NAD) synthesis in the pathogenesis of *Coxiella burnetii*

Mebratu A Asaye[1], **Chen Ai Khoo**[2], **Nitika Neha**[1, 3], **Dedreia Tull**[3], **David P De Souza**[3], **Nadeeka K Wawegama**[1], **Hayley J Newton**[2], **Fiona M Sansom**[1]

1. Asia-Pacific Centre for Animal Health, Melbourne Veterinary School, The University of Melbourne, Parkville, VIC, Australia

2. Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Parkville, VIC, Australia

3. Metabolomics Australia, Bio21 Institute of Molecular Science and Biotechnology, The University of Melbourne, Parkville, VIC, Australia

Coxiella burnetii, the causative agent of the zoonotic disease Q fever, is an intracellular Gram-negative bacterium. Although improved genetic tools and culturing techniques has recently advanced the study of *Coxiella* pathogenesis, the mechanisms that allow the bacterium to survive and replicate inside the hostile phagolysosome are not well understood. A recent screen of a *Coxiella* transposon mutant library for replication within HeLa cells identified a number of genes required for efficient intracellular replication, including *nadB,* which is predicted to encode L-aspartate oxidase, an enzyme required for de novo nicotinamide adenine dinucleotide (NAD) synthesis. To confirm the role of NadB in intracellular replication, complementation of the *nadB* mutant with a pJB:Kan-3xFLAG plasmid expressing 3xFLAG-NadB was performed. Quantitative and qualitative intracellular replication assays inside HeLa cells conclusively demonstrated that *nadB* is required for intracellular replication. This is in contrast to previous studies of the intracellular pathogen *Mycobacterium tuberculosis*, where the loss of *nadB* appears to be compensated for by salvage pathways, even *in vivo*. To functionally characterise NadB, we used an untargeted metabolomics approach to compare the metabolite profiles of wild-type, mutant and complemented strains. GC-MS and LC-MS analysis revealed key changes in the mutant compared to wild type, with an increase in key pathway metabolites preceding NadB, and a corresponding decrease in downstream metabolites. Bioinformatic analysis of the NadB amino acid sequence revealed the presence of a conserved arginine residue at position 275. Site-directed mutagenesis was performed to mutate this residue to a leucine, which abolishes activity of *E. coli* NadB, and both wildtype and R275L NadB-GST fusion proteins were expressed in and purified from E. coli JM109. Enzyme assays using recombinant wildtype NadB-GST demonstrated typical L-aspartate oxidase activity. Current work is focusing on functional characterization of R275L NadB-GST, to confirm loss of enzyme activity, and complementation of the *nadB* mutant with this mutant protein, to confirm the link between an intact de novo NAD synthesis pathway and intracellular replication.

162

Experimental co-evolution of Yeast

Michael McDonald

Experimental evolution is a powerful method for testing fundamental questions in evolution and ecology. I will present work showing that high-throughput sequencing methods can provide insights into the eco-evolutionary dynamics in experiment populations yeast. In this first study, we carry out whole population sequencing at multiple time points, allowing the tracking of the dynamics of each mutation as it arises during adaptation. Using this sequencing-across-time approach, we reveal that that multiple co-evolving populations emerge spontaneously within evolving lineages. In our yeast experiments, recombination between co-existing sub-populations does not cause the breakdown of the stable co-existence of multiple yeast types. While long-term evolution experiments such as these produce insights, these experiments in laboratory settings are removed from the actual conditions that microbes experience in nature. In an effort to bridge useful experimental models with complex microbial ecologies, we are carrying out long-term evolution experiments with yeast in co-culture with model bacterial species. I will also present data from these on-going experiments.

1. Sex speeds adaptation by altering the dynamics of molecular evolution MJ McDonald, DP Rice, MM Desai Nature 531 (7593), 233
2. Crowded growth leads to the spontaneous evolution of semistable coexistence in laboratory yeast populations EM Frenkel, MJ McDonald, JD Van Dyken, K Kosheleva, GI Lang, ... Proceedings of the National Academy of Sciences 112 (36), 11306-11311

163

Mucormycosis in the platypus and the amphibian

Joanne H Connolly[2, 1], Seyed Ali Ghorashi[2, 1], Benjamin J Stodart[2, 3]

1. School of Animal and Veterinary Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

2. Graham Centre for Agricultural Innovation, Wagga Wagga, NSW, Australia

3. School of Agricultural and Wine Sciences, Charles Sturt University, Wagga Wagga, NSW, Australia

Mucormycosis in the platypus and the anuran (frogs and toads) is a major fungal disease caused by the dimorphic Mucorale, *Mucor amphibiorum*. First reported from a German Zoo in 1972, *M. amphibiorum* infection resulted in a disseminated mycosis in a green tree frog imported from Australia. Since 1994, naturally occurring infections in wild anurans from Queensland and Northern Territory, and captive frogs from Melbourne and Perth have been recorded. A severe ulcerative skin condition was first reported in platypuses from the Elizabeth River in Tasmania in 1982, however the causative agent was not confirmed as *M. amphibiorum* until a decade later. The granulomatous and ulcerative dermatitis may progress to involve underlying muscle or disseminate to internal organs. The sudden emergence of mucormycosis in Tasmanian platypuses may have been by accidental introduction with 'banana box frogs' from Queensland or due to an endemic Tasmanian strain that mutated becoming pathogenic for platypuses. Only positive mating types have been isolated from Tasmanian platypuses, while both mating strains have been isolated from mainland anurans. The ecologic niche of *M. amphibiorum* on the mainland is soil and anurans, whereas in Tasmania (other than platypus lesions) its niche is currently unknown.

Differentiating species of *Mucor* was traditionally based on phenotypic characters. More recently genotypic analysis has been shown to be reliable and discriminating. Isolates of *Mucor* sp. were genotyped by sequencing of the internal transcribed spacer (ITS) region, followed by inter-simple sequence repeats polymerase chain reaction (ISSR-PCR) for diversity analysis. Recently, PCR followed by high-resolution melt (HRM) curve analysis has been used for detection and/or genotyping of a variety of microorganisms including filamentous fungi. We investigated a PCR targeting ITS regions of rDNA genes and HRM analysis for subsequent *Mucor* species identification.

The use of evolving molecular tools to detect *M. amphibiorum* in the environment, tissue lesions, and aquatic vectors, would improve our understanding of mucormycosis epidemiology, leading to better surveillance and control.

Oral Abstracts

164

Tropical fungal endophytes, mountain islands and host preference

Kaylene Bransgrove[2, 1], Darren Crayn[1], Sandra Abell[1]

1. Australian Tropical Herbarium, Cairns, Qld, Australia

2. QDAF, Brisbane, QLD, Australia

Fungal endophytes are a highly diverse group of fungi that asymptomatically inhabit all plant parts. Their beneficial impact on plant health is well documented and development of the anti-inflammatory or antibiotic-like secondary metabolites they produce for agricultural and human health purposes is a growing field of research. Little is known regarding their diversity across the Australian landscape or their host specificity in tropical trees.

Leaf material was collected from *Elaeocarpus carolinae* from mountain-tops across the Wet Tropics of northern Queensland and from *Elaeocarpus grandis*, *Endiandra microneura* and *Normanbya normanbyii* from a lowland site in Cape Tribulation, northern Queensland. Whole genomic DNA was extracted from surface sterilised leaves and the fungal component sequenced using amplicon next generation sequencing. Differences between sites and hosts were assessed and visualised using permutational anova and constrained ordination respectively.

Fungal endophyte communities were significantly different between mountain top sites (*E. carolinae*) and between tree hosts (*E. grandis, E. microneura* and *N. normanbyii)*. Some endophyte communities were more similar to those from nearby mountain tops than distant mountains and communities of *E. grandis* and *N. normanbyii* were more similar to each other than to *E. microneura*. These results show tree host and locality do influence fungal endophyte communities but a broader sampling of hosts and localities is necessary to better understand the relationship between host genetic diversity and the composition of fungal endophyte communities across the landscape.

165

Copper (II) Lead (II) and Zinc (II) reduce the growth, zoospore production and attachment rate to organic substrates of three species of zoosporic fungi from soils of NSW

Linda Henderson[1], Agostina V Marano[2], Frank H Gleason[1], Osu Lilje[1], Elayna Truszewskia[1]

1. School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW, Australia

2. Núcleo de Pesquisa em Micologia,, Av. Miguel Estéfano, Instituto de Botânica, São Paulo, SP, Brazil

Zoosporic true fungi (chytrids) are common and widely distributed in soils as saprotrophs and parasites. They attach to, and grow on many substrates of plant and animal origin, such as pollen, keratin and chitin. Three saprotrophic isolates of zoosporic fungi from the soils of NSW are examined here: *Rhizophlyctis rosea* (A13) from Sydney, *Chytriomyces hyalinus* (A14) from the Central coast and *Gaertneriomyces semiglobifer* (Mar-CC2) from Narrabri, for their ability to reproduce (produce zoospores), grow (increase in biomass) and adhere to cellulose and chitin in the presence of soluble Copper (II), Lead (II) and Zinc (II). All isolates declined significantly in growth at 60 ppm (0.94 mmol m^{-3}) Cu, three declined significantly at 60 ppm (0.92 mmol m^{-3}) Zn and two declined significantly at 100 ppm (0.48 mmol m^{-3}) Pb. All isolates declined in zoospore production at 60 ppm Cu, three at 60 ppm Zn and three at 100 ppm Pb. Two isolates declined significantly in attachment rate at 60 ppm Cu, two at 60 ppm Zn and two at 100 ppm Pb. Rhizoids of one isolate significantly increased in number and length when incubated with 20, 30 and 60 ppm Pb. If similar effects are caused by these metals in soils, Cu, Pb and Zn contamination of NSW soils is likely to reduce the growth, reproduction and attachment of zoosporic true fungi; thereby reducing the rate of mineralisation of soil organic matter.

166

Fermenting Yeasts and Where to Find Them

Edward D Kerr[1], Mark T Howes[2], Benjamin L Schulz[1]

1. University of Queensland, Brisbane, QLD, Australia

2. Newstead Brewing Company, Brisbane, QLD, Australia

Yeast is essential for making beer, as it produces ethanol and contributes heavily to beer's flavour and sensory properties. Commercial breweries typically use *Saccharomyces cerevisiae* for production of ales, and *Saccharomyces pastorianus* for lagers. The most notable exceptions to this are sour, lambic, and American coolship ales, where wort is exposed to a mix of environmental yeast and bacteria, rather than commercially cultivated *Saccharomyces* strains. Spontaneous environmental inoculation for beer fermentation is becoming more common and rather trendy. However, such wild ferments have unpredictable rates, efficiencies, and end products. We propose instead to isolate clonal strains of wild yeast, to allow efficient and predictable production of beers with interesting and diverse flavour profiles. Here, we describe a method for isolating and characterising wild yeast for brewing unique and interesting beers. Yeast is collected from the wild and grown on solid selective media to isolate clonal strains. These clones are identified by internal transcribed region (ITS) PCR and Sanger sequencing. Each clone is grown in liquid wort to measure growth on oligomaltose, and to identify fermentation end products. Gas chromatography mass spectrometry (Headspace-GCMS) is then used to quantify ethanol and other fermentation end products from fermentation. Strains that efficiently grow and produce ethanol are then fermented at 10ºC and 20ºC, and flavour profiles are measured using Headspace-GCMS. Intermediate upscaling of strains with efficient growth, ethanol production, and pleasant or interesting smelling ferments is then performed in a food grade brewery to allow sensory evaluation. This workflow provides the tools needed go from environmental yeast isolation to producing brewery-quality beer. This workflow also has uses beyond brewing, allowing quick and robust identification of wild yeasts and identification of compounds related to flavours in diverse beverages or industrial applications.

Oral Abstracts

167

Identifying changes in painted and polyester surfaces during the attachment and proliferation of three species of environmental fungi using Synchrotron-sourced macro ATR-FTIR microspectroscopy

Vi Khanh Truong[1], Stephanie Owen[1], Jitraporn Vongsvivut[2], Mark J Tobin[2], Shane MacLaughlin[3], Russell Crawford[4], Elena P Ivanova[4]

1. Swinburne University of Technology, Melbourne, VIC, Australia

2. Infrared Microspectroscopy (IRM) Beamline, Australian Synchrotron, Clayton, VIC, Australia

3. BlueScope Steel Research, Port Kembla, NSW, Australia

4. School of Science, RMIT, Melbourne, VIC, Australia

Fungi are able to colonise and grow on many surfaces, including painted surfaces, under a variety of environmental conditions. This leads to surface deterioration and discolouration, eventually requiring costly repair or replacement. The development of surfaces that resist fungal colonisation is inhibited by a lack of research and understanding into the specifics of fungal attachment strategies. In this work, the attachment and proliferation of three characteristics fungal species found in indoor and outdoor environments are studied: *Aspergillus niger* ATCC 9642, which is particularly prevalent indoors; *Aureobasidium pullulans* ATCC 42773, the primary coloniser for paint in humid conditions and *Epicoccum nigrum* ATCC 9348, which has been identified as a coloniser of polymer-coated roof surfaces. Each species was incubated on either polymer-coated steel discs or the same steel discs covered with Interior Taubmans® Easycoat paint. Field-emission SEM was used to quantify cell attachment after 1, 3 and 7 days of incubation. Synchrotron-sourced macro ATR-FTIR microspectroscopy was used to analyse chemical changes in the fungi and the surfaces as they attached and began to hyphenate. Microscale changes were identified related to fungal hypha, with the degree of change related to the species' metabolic competency. Using macro ATR-FTIR microspectroscopy, it was found that the polysaccharides were detected at a maximum amount during early colonisation, suggesting that secreted polysaccharides may play an important role during initial fungal surface attachment. By identifying changes in fungal and surface chemistry during attachment and proliferation, we were able to identify potential attachment mechanisms of interest for future research. This will assist in developing surfaces that resist fungal colonisation and degradation.

168

Fungal Pathogen-Host Interactions – The Winner Takes It All

Karl Kuchler[1]

1. Medical University Vienna, Vienna, Austria

Fungal pathogens are considered hidden killers of mankind, as invasive fungal infections claim around 1,5 million lifes per year. Thus, fungal infections constitute a medical problem of epic proportions. The most prevalent fungal pathogen Candida albicans represents the 4th-most frequent cause of hospital-acquired infections with an overall mortality rate of about 40%. Antifungal immunity is driven by a dynamic and complex interplay of innate and adaptive immunity, particularly engaging T cell-mediated inflammatory defense at later stages of infections. The level and amplitude of host inflammation is critical for the outcome of invasive fungal diseases. In my lecture, will discuss how host invasion by Candida spp can trigger cell-type specific protective as well as detrimental immune responses. In addition, I will also discuss some of the mechanisms that Candida spp exploit to evade immune surveillance and thus escape clearing by immune cells. Finally, I shall discuss an emerging paradigm change and novel approach for the treatment of invasive fungal diseases, which is to modulate the host immune response to fungal pathogens rather than targeting the pathogen itself.

201

Evaluation of *EasyScreen™* ESBL/CPO Detection Kit using direct-PCR from patient culture and broth samples

Dilshan M Abeysekera[1], Doug S Millar[1], John R Melki[1], Elaine McGrath[2], Martin Cormican[2]

1. Genetic Signatures, Newtown, NSW, Australia

2. Division of Microbiology, University Hospital Galway, County Galway , Ireland

Background

Beta-lactam and carbapenem antibiotics are the most commonly used worldwide in the treatment of bacterial infections. The recent emergence of ESBL and CPO is a significant global concern in healthcare settings as standard treatments may be rendered ineffective. Thus accurate and rapid detection of these resistant organisms will have a significant impact on patient management. We evaluated a *EasyScreen™* ESBL/CPO Detection Kit to detect most significant and commonly encountered bacterial resistance genes TEM, CTX-M, SME, GES, IMP, NDM, OXA-23 like, OXA-48 like, OXA-51 like, MCR-1, DHA, SHV, VIM, IMI, CMY, KPC and their subtypes in patient culture and broth samples.

Methods/Materials

To test the *EasyScreen™* ESBL/CPO Detection Kit's sensitivity/specificity synthetic DNA constructs, validation organisms and panels from Vircell, Zeptometrix and QCMD were used. The assay was performed on 30 known clinical isolates from University Hospital Galway. 9 isolates were re-cultured in BHI-broth (with Ceftazidime). Inoculated samples were lysed/converted at 95°C for 15 minutes and directly amplified on the CFX 96/384 PCR-machines and compared with data from conventional DNA extracted and amplified samples.

Results

Results from the validation panels yielded 100% concordance with the expected resistance patterns. The agreement with known clinical samples was 98%. Mixed infections could be easily detected using the different channels of the PCR instrument.

Conclusions

The *EasyScreen™* ESBL/CPO Detection Kit can be used to detect resistant genes directly from cultures and broths without the need for a DNA extraction/purification step. The PCR protocol can be significantly manipulated to reduce run-time given the high copy number. Turn-around-time is approximately 2hr for cultures and 1hr for broths.

The 3base™ technique expands the detection capacity of multiplex-PCR for some target genes (CTX-M, IMP-14) to detect various subtypes within the target by affecting the DNA sequence homology. Also, novel variants or new resistant markers can readily be incorporated into existing assays easily given the properties of the 3base™ converted DNA, thus improving the coverage of such assays. This assay provides a rapid, sensitive, and cost-effective alternative for the detection of ESBL and CPO's.

202

Role of flagella as an immune modulator during urinary tract infection

Dhruba Acharya[1], Benjamin Duell[1], Matthew Sullivan[1], Mark Schembri[2], Glen Ulett[1]

1. Griffith University, Southport, QLD, Australia

2. University of Queensland, Brisbane, QLD, Australia

Urinary Tract Infections (UTIs) are a huge public health problem affecting 150 million people each year worldwide. UTIs are predominantly caused by uropathogenic *Escherichia coli* (UPEC). The roles of flagella in the pathogenesis of UPEC UTI, including immune responses to infections are not well understood. A protective role for IL10 in acute UTI has been shown; however, the UPEC virulence factor(s) responsible for IL10 induction is yet to be identified.

We hypothesised that UPEC flagella induce the production of IL10 during acute UTI. UPEC CFT073 and a derivative strain containing four mutations in genes encoding fimbriae and pili (CFT073-Quad) were deleted for the flagellar filament, *fliC*, using overlap-extension PCR and gene replacement with λ-red recombinase. The *flhDC* gene, which regulates flagella biosynthesis, was used in IPTG-inducible pMG600 to overexpress flagella. Extraction and purification of flagellar filaments was performed using a combined ultracentrifugation and Fast Performance Liquid Chromatography (FPLC) approach. Endotoxin was removed using commercial columns to purify the flagellin to homogeneity. Monomerisation assays were performed using temperature gradients between 50°C and 90°C and proteins were visualized in native gels. Flagellin concentration and purity were determined using BCA, coomassie-stained SDS-polyacrylamide gels, western blotting and mass spectrophotometry. Immune modulation by flagellin was assessed using a co-culture model of 5637 uroepithelial cells and U937 monocytes to measure cytokine responses, including IL10 following challenge with either WT, a-flagellate, or hyper-flagellate UPEC CFT073, or purified flagellin protein.

Flagellin protein induced IL10 in addition to several other cytokines in the co-culture model. Knowledge of how Flagellin acts as an immune modulator during UTI, based on the analysis of highly purified protein, could offer new avenues for infection prevention or control.

Poster Abstracts

203

A Comparison of the Results of Three Methods of MIC Determination

Julie K Allerton[1], Dianne L Rafferty[1], Pratibha M James[1], Sydney M Bell[1]

1. CDS Reference Laboratory, NSW Health Pathology, Kogarah, NSW, Australia

The purpose of antimicrobial susceptibility testing (AST) is to provide a confident prediction of the likely outcome of treatment. Methods calibrated to quantitative susceptibilities provide a means for diagnostic laboratories to deliver such predictions. In order to ensure comparable and reliable results, ISO 20776-1 advises that routine methods and diagnostic devices be evaluated against its reference broth microdilution method. While the three most commonly adopted disc diffusion methods in Australia differ marginally in selection of agar, disc potency and inoculum, CDS, CLSI and EUCAST can be related to each other because they are all calibrated against minimal inhibitory concentration (MIC).

The MICs of five international reference strains was determined by agar dilution on Sensitest and Mueller-Hinton agars using a Steer's replicator and by the ISO broth microdilution method. Each technique of susceptibility testing has relative advantages and disadvantages and may be affected by reagent deterioration, minor technical deviations or subjectivity in end-point reading.

We compare the MIC results observed with three methods. Results were considered concordant if they fell within ± 1 doubling dilution of each other and within the published range for the organism.

204

A Review of Polymyxin Disc Testing from a Calibrated Dichotomous Susceptibility Perspective

Julie K Allerton[1], Dianne L Rafferty[1], Pratibha M James[1], Sydney M Bell[1]

1. CDS Reference Laboratory, NSW Health Pathology, Kogarah, NSW, Australia

With the increasing worldwide prevalence of multi-drug resistant (MDR) Gram negative bacteria, polymyxin has re-emerged as a last resort treatment. Polymyxin is a lipopeptide antibiotic isolated from *Bacillus polymyxa*.

In 1975 the Calibrated Dichotomous Sensitivity (CDS) method recognised that polymyxin B had poor diffusibility in agar resulting in zones smaller than the standard 6mm annular radii. Thus, interpretation of polymyxin is based on an annular radius of 4mm or more for susceptibility, equivalent to a minimal inhibitory concentration of less than or equal to 1.0mg/ml.

Antibiotic disc susceptibility testing remains the most widely used method in clinical laboratories, but recently the accuracy of disc testing methods for polymyxins has come under scrutiny. While resistance to the agent may be intrinsic, chromosomally encoded or plasmid mediated, acquired resistance is still quite rare.

In recent times there has been numerous publications denouncing disc diffusion testing for the polymyxin family. Many studies have focused on colistin (polymyxin E) and Mueller-Hinton agar. Poriel et al observed that Iso-sensitest agar was more sensitive than Mueller-Hinton in detecting resistant subpopulations of *Enterobacter cloacae* isolates. Sensitest agar used in CDS more closely resembles the composition of Iso-sensitest than Mueller-Hinton and in combination with the use of polymyxin B 300u discs may offer an advantage over other disc diffusion methods.

This review was undertaken to establish whether CDS users can continue to be confident in reporting polymyxin B according to their method. The study examined two aspects. First, an analysis of polymyxin B results from three major public hospitals in metropolitan and south eastern New South Wales. Data from 2007 - 2017 was assessed for prevalence of resistance. The second part of the evaluation involved a comparison of minimal inhibitory concentrations (MIC) determined by agar diffusion with disc diffusion and broth microdilution MICs.

205

Genetic diversity and distribution of filamentous phages in *Neisseria*

Barakat Alsuwayyid[1, 2], Michael Wise[3], Geoffrey Coombs[4, 5], Julie Pearson[4], David Speers[7, 6], Leah Rankine-Wilson[1], Charlene Kahler[1]

1. School of Biomedical Science/Division of Infection and Immunity, The University of Western Australia, Perth, WA, Australia

2. Ministry of Education, Riyadh, Saudi Arabia

3. Computer Science and Software Engineering, The University of Western Australia, Crawley, WA, Australia

4. Department of Microbiology, Pathwest Laboratory Medicine WA, Murdoch, WA, Australia

5. School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia

6. School of Medicine and Pharmacology, The University of Western Australia, Crawley, WA, Australia

7. Department of Microbiology, Pathwest Laboratory Medicine WA, Nedlands, WA, Australia

A filamentous bacteriophage termed the Meningococcal Disease Associated (MDA) phage is associated with *Neisseria meningitidis* clades which cause invasive meningococcal disease. MDA phage improves mucosal colonization of the nasopharynx by meningococci and thus increasing the incidence of bloodstream invasion associated with meningococcal carriage. We recently recovered a gonococcal isolate (ExNg63) from a rare case of gonococcal meningitis and whole genome sequencing revealed that this isolate possessed a region with 90% similarity to the MDA phage found in *N. meningitidis*. Sanger sequencing confirmed that the entire MDA-like phage was intact in the genome of ExNg63. This is the first indication that MDA-like phages may not be restricted to *N. meningitidis*. Therefore, to understand the genetic diversity and distribution of MDA-like phages, we examined the distribution, prevalence and genetic diversity of MDA-like phages in Neisseriaceae.

Closed genomes of 44 *N. meningitidis*, 28 *N. gonorrhoeae*, 2 *N. lactamica* and 17 commensal *Neisseria* species were collected from the NCBI database and BIGSdb. Filamentous prophages were defined as a set of genes that have the size and genetic organization similar to the MDA phage in *Neisseria*

meningitidis Z2491 or Ngo6-8 in *Neisseria gonorrhoeae* FA1090. A maximum likelihood phylogenetic tree was constructed using MEGA7 with 500 bootstrap replicates while heirBAPS was used to define genetic population groups of prophages using MAFFT aligned prophage sequences.

One hundred and sixty filamentous prophages were detected in the dataset and population structure analysis using heirBAPS revealed that the putative gonococcal MDA-like phages and a putative MDA-like phage in *N. lactamica* formed a structure group with meningococcal MDA-phage. However, only 292 of 3800 gonococcal isolates available at BIGSdb possessed a complete or partial MDA-like sequence suggesting that acquisition of MDA-like phages is rare in this species. These data suggest that prophages similar to the meningococcal MDA phage are present in *N. gonorrhoeae* and *N. lactamica* and more work is required to determine whether MDA-like phages act as accessory colonization factors in these species.

206

Establishment of bacterial microbial biofilms from Antarctic soil bacteria.

Fabian Davamani Amalraj[1], Chong Sze Ling Catherine[1], Chong Han Lung Daniel [1], Nur E'zzaty Nadhirah Mohamad[1], Sanjeni Selvarajah[1], Stephen Ambu[1], Wan Loy Chu[1, 2], Pete Convey[3]

1. International Medical University, Bukit Jalil, KUALA LUMPUR, Malaysia

2. 2National Antarctic Research Centre, University of Malaya, Kuala Lumpur, Malaysia

3. British Antarctic Survey , Cambridge, CB3 0ET, United kingdom

The Antarctic soils are exposed to a wide range of environmental factors which can limit or promote the survival of psychrophilic/psychrotolerant microbes. Biofilms are the dominant life form which can withstand stressful conditions while biofilm-forming ability is an important survival strategy of microbial community. The physical extremes of temperature, ultraviolet (UV) radiation, salinity, low water and nutrient availability are amongst the growth-limiting factors of Antarctic bacteria. Temperature and nutrition were the growth-limiting parameters selected as the focus of the current study. The primary aim of this study was to assess the effects of temperature and nutrients on the growth of Antarctic bacterial biofilms. The soil samples from Signy Island were used to extract the microbes after vortexing and slow -speed centrifugation and plated on 24 different microbial media, including defined media and complex medium. The cultures were incubated at 4˚C, 10˚C and 15˚C for a period of 15-30 days and observed for the formation of biofilms. The biofilms formed were estimated by crystal violet method. Our experimental study highlighted that soil samples showed varied degree of growth. Simple descriptive statistics were used to describe the overall trend of biofilm growth together with the use of median absolute deviation to facilitate the identification of outliers in each data set. The data were non-normally distributed and positively skewed. Therefore, inferential statistics was used to evaluate the success in biofilm formation. Bootstrap method was used, where each data set was resampled and the mean confidence interval was determined.

207

Quality assurance program for molecular detection of gastrointestinal parasites: results of pilot module.

Norvie L Aquino[1], Elizabeth Haremza[1], Debra Walker[1], Michelle Bullivant[1], Allan Elsner[1]

1. Microbiology, The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), St Leonards, NSW, Australia

Background:

Parasitology laboratory routine diagnosis involves traditional morphological identification of parasites by microscopy and staining. Recently, molecular methods are increasingly used by more laboratories to enhance the identification of parasites. The demand to start a proficiency testing program is due to the emergence of these molecular methods in the absence of an existing QAP and, in part because of the need for faster reporting and diagnosis. In response to this, The RCPA Quality Assurance Programs developed a pilot survey to assess the detection and identification of gastrointestinal parasites.

Materials/methods:

The first pilot study of the gastrointestinal parasites module included six simulated clinical specimens:

- Two samples were prepared in fixed stool and washed three times with saline, one contained *Giardia* species and *Cryptosporidium* species (**A**) whilst the other had *Dientamoeba fragilis* (**C**).
- One negative that contained only a faecal matrix (**B**).
- One was a commercial sample of *Entamoeba histolytica* (**D**).
- Two fresh stool samples with DNA stabiliser had *Blastocystis hominis* (**E**) and *Giardia* species (**F**) respectively.

Graphs and tables were used to show results according to the methods and investigations used by the participants.

Results:

Across the 28 participating laboratories from Australia and globally, the results were returned as follows:

- Two participants reported *D. fragilis* on the sample that had *Giardia* species and *Cryptosporidium* species (**A**) whilst 26 participants reported "no parasites detected".
- *D. fragilis* (**C**)was correctly reported by one participant whilst 27 reported "no parasites detected".
- The negative sample (**B**) was reported as "no parasites detected" by all participants (100%).
- Twenty-seven (96%) participants reported *Entamoeba histolytica* (**D**).
- Nine participants reported *B. hominis* (**E**) present.
- Twenty-eight (100% concordance) participants reported *Giardia intestinalis* (**F**).

Five participants used in-house assays, whilst the remaining 23 used six different commercial amplification/detection platforms. PCR assay controls (inhibition, negative and positive) were employed by fourteen (50%),12 (43%) and 14 (50%) participants respectively.

Conclusion:

The molecular gastrointestinal parasite pilot module QAP had enabled participating laboratories to review the pre-analytical, analytical and post-analytical stages of their molecular testing protocols. This also created a positive effect on participants by influencing changes and updates to their assays, algorithms and reporting protocols.

208

New insights into the functional roles of DNA methylation in extraintestinal pathogenic *Escherichia coli*.

Melinda M Ashcroft[1, 2, 3], Brian M Forde[1, 2, 3], Minh Duy Phan[1, 2], Kate M Peters[1, 2], Mark A Schembri[1, 2], Scott A Beatson[1, 2, 3]

1. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

2. Australian Infectious Diseases Research Centre, The University of Queensland, St Lucia, QLD, Australia

3. Australian Centre for Ecogenomics, The University of Queensland, St Lucia, QLD, Australia

DNA methylation, catalysed by DNA methyltransferases (MTases), guides numerous biological processes including genomic defence against foreign DNA, DNA replication and repair, transposition and regulation of gene expression and virulence[1]. MTases are often encoded on mobile genetic elements (MGEs), which have a key role in *E. coli* evolution. Third generation sequencing technologies allow us to *de novo* assemble complete genomes, resolve complex MGEs and define the genome-wide complement of DNA methylation (methylome). Our investigations reveal several distinct narratives on how the methylome may contribute to the evolution of extraintestinal pathogenic *E. coli* (ExPEC).

(i) In *E. coli* Sequence Type (ST)101, we showed strain-specific methylation differences, where an additional MTase encoded on a MGE resulted in differential methylome patterns within the promoter regions of 31 genes, suggestive of functional differences between two almost identical strains.

(ii) In ST101, we also showed how recombination events at a single genomic locus encoding a restriction-modification system methylating approx. 500 sites throughout the genome, caused global methylome changes within the lineage.

(iii) In the ST131 strain EC958, we found that the distribution of MTase sites is not random between the core and accessory genome. For Dam methylation, this difference is partially accounted by large (>=1 kbp) methylation-free regions in MGEs, suggestive of evolutionary pressure and selection against Dam methylation in MGEs.

(iv) Investigations of knockout mutants of global regulators in EC958 showed that transcription factors have a much larger role in the regulation of *E. coli* methylation than previously thought, resulting in both repression and activation of EC958 MTases.

Our findings suggest numerous possible functional roles for DNA MTases in ExPEC, particularly genome-wide gene regulation. These studies highlight the need for fine-scale genomic and methylome characterisations for a full understanding of the impact of DNA methylation on ExPEC evolution.

1. Casadesús J and, Low D. Epigenetic gene regulation in the bacterial world. Microbiol Mol Biol Rev. 2006. 70(3):830-56.

209

Lipid A profiling and metabolomics of polymyxin-susceptible (PB^S) and -resistant (PB^R) MDR *Klebsiella pneumoniae*

Su Aye[1], Irene Galani[2], Meiling Han[1], Darren J. Creek[3], Ilias Karaiskos[4], Yan Zhu[1], Eleni Giamarellou[4], Tony Velkov[5], Jian Li[1]

1. Monash Biomedicine Discovery Institute, Department of Microbiology, Monash University, Melbourne, Victoria, Australia

2. Fourth Department of Internal Medicine, National and Kapodistrian University of Athens, Athens , Athens, Greece

3. Drug Development and Innovation, Drug Delivery, Disposition and Dynamics, Monash Institute of Pharmaceutical Sciences, Melbourne, Victoria, Australia

4. Sixth Department of Internal Medicine, Hygeia General Hospital, Athens, Greece

5. Department of Pharmacology & Therapeutics, School of Biomedical Sciences , Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Victoria, Australia

ABSTRACT

The rapid increase of polymyxin-resistant MDR *K. pneumoniae* isolates has become a major global concern. This study aimed to investigate lipid A profiles and metabolomes of PB^S and PB^R MDR *K. pneumoniae*. Three paired of clinical isolates (Kp AHT 8 [PB MIC 64mg/L] vs AHT 7 [0.25mg/L], Kp AHT 16 [32mg/L] vs AHT 15 [0.5mg/L], and Kp AHT 18 [64mg/L] vs AHT 17 [1mg/L] were examined using liquid chromatography mass spectrometry and differences in the lipid A profiles and metabolomes were identified through multivariate and univariate statistics. The predominate forms of lipid A in these paired isolates were hexa-acylated, and lipid A species from PB^R isolates were mostly modified with 4-amino-4-deoxy-L-arabinose. The metabolomic profiles of PB^S and PB^R isolates were significantly different. In detail, UDP-*N*-acetyl-D-glucosamine, UDP-*N*-acetyl-D-mannosaminuronate associated with lipid A biosynthesis were significantly depleted in all PB^R isolates (>1.5 FC, $p < 0.05$), compared to their parent isolates. The intermediate metabolites in carbohydrate metabolic pathway, particularly pentose phosphate pathway (PPP) and tricarboxylic acid (TCA) cycle, were significantly lower in all PB^R isolates (approximately >1.5 FC, $p < 0.05$). Most nucleotide metabolites were also lower in abundance in PB^R isolates. Two important metabolites associated with the glycerophospholipid (GPL) metabolism, *sn*-glycerol-3-phosphate and *sn*-glycero-3-phosphoethanolamine were significantly lower in abundance in all PB^R isolates (> 2 FC, $p < 0.05$). Remarkably lower GPL levels were evident in PB^R AHT 16 and AHT 18 (approximately >1.5 FC), compared to their parent isolates, while no significant GLP changes were observed in PB^R Kp AHT 8. This study is the first to reveal that polymyxin resistance causes different metabolism in PB^R MDR *K. pneumoniae* and provided valuable mechanistic information for the development of rational polymyxin combinations against PB^R*K. pneumoniae*.

210

Genomic and transcriptomic analysis of nontypeable *Haemophilus influenzae* from paediatric chronic lung disease patients

Ammar Aziz[1], Derek S. Sarovich[1, 2], Elizabeth Nosworthy[1], Jemima Beissbarth[1], Anne Chang[1], Heidi Smith-Vaughan[1], Erin P. Price[1, 2], Tegan M. Harris[1]

1. Menzies School of Health Research, Tiwi, NT, Australia

2. Faculty of Science, Health, Engineering and the Environment, University of the Sunshine Coast, Sunshine Coast, QLD, Australia

Non-typeable *Haemophilus influenzae* (NTHi) is a commensal bacterium of the upper respiratory tract of healthy children. On occasion, NTHi can infect the lower airways, driving early-stage chronic lung disease. The molecular basis underpinning NTHi transition to a pathogenic lifestyle is poorly understood. Here, we performed comparative genomic and transcriptomic analysis of 12 paired isogenic NTHi strains isolated from the nasopharynx (NP) and bronchoalveolar lavage (BAL) of 11 children with bronchiectasis or chronic suppurative lung disease. Our objective was to identify convergent molecular signatures associated with lung adaptation. Genomic analysis identified between one and six mutations separating NP and BAL pairs, with one-third causing substitutions or deleterious frameshifts, and another third occurring in probable promoter regions. Within-patient analysis detected differentially expressed (DE) genes associated with virulence, immune evasion, epithelial cell adhesion and biofilm formation. Comparison of NP and BAL transcriptomes failed to identify signatures of convergence. However, functional enrichment analysis of within-patient DE genes revealed that lung isolates were significantly under-represented in genes involved in translation, ribosomal structure and biogenesis pathways and of unknown function, and significantly over-represented in cell motility, secretion, and carbohydrate transport/metabolism categories. This observed trend in genetically-unrelated NTHi strains suggests that pathogen factors play a role in airway adaptation. Our results provide an important first glimpse into the molecular adaptation of NTHi to paediatric airways. Understanding these pathoadaptative mechanisms is essential for devising targeted treatments aimed at minimising exacerbation severity, and ultimately, preventing NTHi lung infections and subsequent chronic lung disease.

211

Antimicrobial mechanisms of arachidonic acid at the host-pneumococcal interface

Stephanie L Begg[1], Bart A Eijkelkamp[1], Victoria G Pederick[1], Claudia Trapetti[1], Melissa K Gregory[2], James C Paton[1], Christopher A McDevitt[1]

1. Research Centre for Infectious Diseases, University of Adelaide, Adelaide, South Australia, Australia

2. Adelaide Medical School, University of Adelaide, Adelaide, South Australia, Australia

Streptococcus pneumoniae (pneumococcus) is a significant global pathogen responsible for more than 1 million deaths every year. To survive within the human host, this pathogen must evade both innate and adaptive immunity to proliferate and survive. As part of the innate immune response, the human host utilises long chain fatty acids, such as the polyunsaturated fatty acid, arachidonic acid (AA), in the defence against bacterial infection. However, the mechanisms by which AA contributes to bacterial clearance are not well understood.

In this study, we applied a murine model of pneumococcal infection to investigate the contribution of AA to antimicrobial defence. Here, we show that the concentration of AA increased 44% in response to *S. pneumoniae* infection, suggesting a role in innate immune defence against the pneumococcus. The antimicrobial activity of AA was then analysed using THP-1 macrophages. This revealed that supplementation with AA increased pneumococcal killing by 50%, compared to un-supplemented macrophages. We then conducted RNA sequencing of *S. pneumoniae* in the presence of mild AA stress to determine the mechanism(s) of AA antimicrobial activity. These results show that AA stress caused ~4-fold down-regulation of the fatty acid biosynthesis gene cluster, with subsequent membrane composition analyses revealing a significant reduction in the abundance of endogenously produced fatty acids. Further, the abundance of AA in the membrane increased by 31%, leading to changes in membrane fluidity and integrity.

Collectively, these data show that AA is selectively increased in host serum in response to pneumococcal infection. Increased AA abundance contributes to bacterial clearance through direct antimicrobial activity, predominately mediated though disruption of the pneumococcal membrane via concomitant down-regulation of endogenous fatty acid production and the physical insertion and accumulation of an exogenous fatty acid within the pneumococcal membrane.

212

Identifying persistent E. coli strains to harsh life outside the host

Mahboobeh Behruznia[1], David Gordon[1]

1. Research School of Biology/ Division of Ecology and Evolution, The Australian National University, Canberra, ACT, Australia

Escherichia coli has long been used as an indicator of recent faecal contamination in a water body. However, little is known concerning the extent to which conditions in the external environment shape the genetic structure and diversity of *E. coli* communities in the species' secondary habitat. Septic tanks represent a good system with which to investigate *E. coli* community structure in environments external to the host. For a given septic tank, the source of the faecal inputs are largely unambiguous and temporally, relatively constant. Yet the physio-chemical conditions in the septic tank will vary seasonally, with the type of septic system, and with the exact nature of the non-faecal inputs.

Samples were taken from 66 septic tanks on rural residential properties in the Canberra region of NSW, Australia. Samples were processed and plated onto MacConkey agar and up to 44 isolates were characterised per tank. Phylogenetic group membership of isolates was determined by quadruplex PCR and genetic diversity was determined by rep-PCR fingerprinting. Physical and chemical analyses included temperature, pH, and conductivity; as well, ammonium, nitrate, phosphate, and surfactant concentrations were determined. Richness (the number of genotypes) and the Shannon diversity index were used to assess the extent to which *E. coli* community diversity varied with the physio-chemical conditions of the septic tank.

More than 700 *E. coli* isolates representing 270 distinct genotypes were recovered. Septic tanks were most frequently dominated by phylogroup A strains (34%), whilst phylogroup B2 strains were only dominant in 19% of the tanks. The median number of distinct genotypes per tank was 2.6 (range 1-16). There was no relationship between the number of people providing inputs to the tank and genotype diversity. Physio-chemical conditions of the septic tanks were found to influence *E. coli* diversity; genotype diversity increased with increasing ammonium concentration, but declined with increasing pH. The results demonstrate that selection is an important determinant of *E. coli* genetic structure in its secondary habitats.

213

Antimicrobial use and resistance in australia (aura) surveillance system: recent developments

John Turnidge[1], Jan Bell[1], Kathy Meleady[1]

1. Australian Commission on Safety and Quality in Health Care, Sydney, NSW, Australia

Objectives: To provide an update on recent developments, and outputs of, the AURA Surveillance System.

Methods: The Commission has established AURA to collect, analyse and provide reports to inform policy and practice.

AURA continues to work with a number of partners to increase data representativeness and analytical power. Funding for Australian Passive Antimicrobial Resistance (AMR) Surveillance, the National Antimicrobial Utilisation and Surveillance Program (NAUSP), the National Antimicrobial Prescribing Survey (NAPS) and the Australian Group on Antimicrobial Resistance is provided through AURA to maintain an integrated and enhanced data capability. Additional data are provided by Sullivan Nicolaides Pathology, the NPS MedicineWise MedicineInsight program, the National Neisseria Network, and the National Notifiable Diseases Surveillance System.

Results: In August 2017, the Commission released the second AURA report[1] which focussed on 2015 data and included 2016 CARAlert data. Highlights from AURA 2017 include:

- Inappropriate prescribing rates in hospitals and the community are approximately 27% and 45% respectively
- Compared with 2014, there was an increase in rates of fluoroquinolone resistance in *Escherichia coli* from blood cultures (+2.5%) and *Shigella sonnei* (+10.9%)
- The proportion of vancomycin-resistant *Enterococcus faecium* isolates from blood cultures in Australia is now higher than that in Europe
- Community-associated MRSA are now a more common cause of *S. aureus* bacteraemia than hospital-associated MRSA.

AURA 2017 highlights areas for action, including:

- Continued monitoring of the spread of resistant strains of *Neisseria gonorrhoeae* to inform treatment guidelines
- For vancomycin-resistant enterococci, strict adherence to infection control guidelines and effective cleaning and sterilisation in healthcare facilities
- Intensifying efforts to reduce unnecessary prescribing in the community and for surgical prophylaxis.

A number of reports have subsequently been released providing detailed analyses of antimicrobial use and resistance including NAUSP, Surgical NAPS, Aged Care NAPS and regular CARAlert updates.

Conclusion: *AURA surveillance* data identify trends in both antimicrobial use and resistance, and highlight areas for improved prevention and control of resistance in Australia.

214

Update on the National Alert System for Critical Antimicrobial Resistances (CARAlert)

Jan M Bell, John D Turnidge, Kim Stewart

Objectives: The National Alert System for Critical Antimicrobial Resistances (CARAlert) was established in March 2016 as part of the Antimicrobial Use and Resistance in Australia (AURA) Surveillance System, managed by the AURA National Coordination Unit. The objective of CARAlert is to provide timely communication on critical antimicrobial resistances (CARs) to state and territory health departments to inform appropriate response strategies.

Methods: CARAlert uses existing testing and confirmation systems to capture data on CARs. Laboratories confirming CARs enter data into the CARAlert database at the time of confirmation. Nominated state and territory health personnel have restricted access to the system and weekly summaries are distributed to them, the Australian Commission on Safety and Quality in Health Care and the Australian Government Department of Health.

Results: From March 2016 to March 2018, over 2,500 CARs have been confirmed. Nationally, carbapenemase-producing Enterobacteriaceae (CPE), either alone or in combination with ribosomal methyltransferases, and azithromycin non-susceptible *Neisseria gonorrhoeae* (ANSNG) were the most frequently reported CARs.

The IMP type CPE, found most often in the *E. cloacae* complex, was the most frequently reported CPE. An outbreak of OXA-48 producing *E. coli* ST38 was reported in Queensland between May 2017 and July 2017. A small cluster of IMP-producing Enterobacteriaceae was confirmed among six neonates in New South Wales.

ANSNG was the most frequently reported CAR from December 2016 to November 2017. Over the six months to March 2018 there was a decline in reports of ANSNG with low-level resistance (MIC < 256 mg/L). An increasing number of ANSNG with high-level azithromycin resistance (MIC > 256 mg/L), and the confirmation of two isolates with both ceftriaxone non-susceptibility and high-level azithromycin resistance is concerning.

There was a peak in reports of multidrug-resistant *Shigella* species in December 2017 and January 2018.
Conclusions: In its second year of operation CARAlert is providing regular and timely antimicrobial resistance data to states and territories and nationally.

215

Introducing Microbial Culture-Metagenome Sequencing (MC-MGS) to characterize gut mucosa-associated microbial communities.

Erwin M Berendsen[1], Emily C Hoedt[1], Amy L Hamilton[2], Jingwan Zhang[3], Fen Zhang[3], Jun Yu[3], Siew C Ng[3], Michael A Kamm[2], Mark Morrison[1]

1. Microbial Biology and Metagenomics, UQ Diamantina Institute - Translational Research Institute, Woolloongabba, QLD, Australia

2. Department of Gastroenterology, St Vincent's Hospital and University of Melbourne, Melbourne, Victoria, Australia

3. Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Human microbiome research has been enabled and empowered by the rapid advances in DNA/RNA sequencing technologies, and provided new insights into the potential roles for the microbiome in health and disease. There has also been a shift in approach, with shotgun metagenome sequencing (MGS) now being adopted to produce an unbiased functional assessment of the microbiota, in comparison to the taxonomic profiling afforded by 16S rRNA gene-amplicon sequencing. However, while MGS is rapidly becoming the preferred approach with samples that are rich in microbial biomass (e.g. stool) constraints remain with the effective use of MGS with DNA from samples with limited microbial density and/or rich in non-microbial DNA. A specific example are the mucosa-associated microbiota (MAM) that reside along the human gastrointestinal tract, which changes in its density, form, and function relative to anatomical site, gender, and/or health status. Here, we will present our development and evaluation of microbe-culture metagenome sequencing (MC-MGS) to better characterise the MAM of Crohn's disease (CD) patients. In this approach, total DNA is extracted from biopsy samples, and a subsample is subjected to the subtractive removal of human DNA using the NEBNEXT protocol; which results in a 2-8 fold enrichment of microbial DNA, as assessed by qPCR. A second matched biopsy that has been cryopreserved in anaerobic buffer is used to inoculate a habitat simulating medium, to produce a MAM-derived consortia and from which metagenomic DNA is extracted and sequenced. Our preliminary results show that the outgrowth cultures show PCR-positivity for fastidious anaerobes such as *Faecalibacterium prausnitzii.* Additionally, we have been able to isolate urease-producing facultative anaerobes from some biopsies. These findings suggest that MC-MGS can provide a useful, additive approach to produce a more detailed, comprehensive and functional understanding of the MAM in patients with CD, and other chronic, non-communicable diseases.

216

Investigating the role of DsbA enzymes in growth and virulence of uropathogenic *Escherichia coli*

Murari Bhandari[1], Rabeb Dhouib[1], Dimitrios Vagenas[1], Makrina Totsika[1]

1. Institute of Health & Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia

Abstract

Antimicrobial resistance (AMR) constitutes a major public health crisis with antibiotic resistant bacterial infections taking 700,000 of lives each year. Emerging AMR is due to the misuse of antibiotics. Unlike antibiotics, anti-virulence drugs aim to disarm bacteria by inhibiting their virulence factors without affecting growth. Disulfide bond formation (Dsb) is critical for protein stability and the catalyst DsbA is considered a promising anti-virulence target as its inhibition will simultaneously inactivate several virulence factors that bacterial pathogens employ during infection. Some pathogenic bacteria, like uropathogenic *Escherichia coli* (UPEC) encode multiple Dsb proteins, but their contribution to biogenesis of different virulence factors is unclear. Our study has investigated the role of DsbA homologues in UPEC growth and virulence in physiologically relevant *in vitro* conditions. We showed that DsbA enzymes are not important for aerobic growth of UPEC strain CFT073 in LB medium. However, in M9 minimal medium, the lack of *dsbA* but not *dsbL* showed significantly reduced UPEC growth. Furthermore, we have shown that DsbA enzymes are essential for functional type 1 fimbriae and flagella expression in UPEC grown in rich and minimal media. Our findings support the notion that inhibition of DsbA will disarm UPEC during host infection and also likely slow down bacterial growth in the urinary tract. Future work aims to assess the full antimicrobial potential of DsbA inhibitors as novel therapeutics for antibiotic-resistant urinary tract infections.

217

Staphylococcus aureus membrane vesicles activate innate immune signalling pathways.

Natalie J Bitto[1], Lesley Cheng[2], Rishi Pathirana[1], Andrew Hill[2], Timothy P Stinear[3], Maria Liaskos[1]

1. Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, VIC, Australia

2. La Trobe Institute for Molecular Science, La Trobe University, Bundoora, VIC, Australia

3. Department of Microbiology and Immunology, University of Melbourne, Parkville, VIC, Australia

Extracellular vesicles are ubiquitously released by all forms of life. The roles of Gram-negative outer membrane vesicles (OMVs) in inter-bacterial communication and host-pathogen interactions are well described in the literature. In contrast, research surrounding Gram-positive membrane vesicles (MVs) is still in its infancy. There is limited knowledge regarding the pathogenic properties of Gram-positive MVs, including their contents, biological functions and role in cellular communication.

This study characterises MVs produced by the Gram-positive pathogen, *Staphylococcus aureus*, and elucidates their ability to induce an innate immune response. Transmission electron microscopy and NanoSight particle tracking analysis (NTA) were used to characterise the amount and size of MVs produced by *S. aureus* at mid-log and early stationary phase. Qubit fluorometric quantification demonstrated that *S. aureus* MVs contain DNA, RNA and proteins. Analysis of MV-derived proteins by Western immunoblot revealed that MVs contain a range of proteins of varying molecular weight. Furthermore, high molecular weight DNA was found to be associated with MVs, while examination of MV-derived RNA using a bioanalyser identified a range of RNA including small RNA. This may suggest that MV-derived RNA has a potential role in regulation of translation of target RNA through RNA-RNA interactions.

Investigation into the immunogenicity of *S. aureus* MVs revealed that they were capable of inducing the production of the pro-inflammatory cytokine interleukin-8 by epithelial cells. Furthermore, using reporter cell lines, we showed that DNA, RNA and lipoproteins associated with *S. aureus* MVs are immunogenic, as they activate Toll-like receptors (TLR) 9, 7 and 2, respectively. This suggests that *S. aureus* MVs may be involved in mediating a pro-inflammatory response in the host through their immunogenic contents.

This study reveals that *S. aureus* MVs contain a range of cargo including nucleic acids and proteins, and may play a role in host immune modulation. The findings facilitate the understanding of a previously uncharacterised mechanism of *S. aureus* pathogenesis and sheds light on the role of MVs in driving inflammation in *S. aureus* infections.

218

Staphylococcaceae and *Pasteurellaceae* found in the oral flora of marsupials and dingoes

Pat Blackall[1], Carmen Espinosa-Gongora[2], Miki Bojesen[2]

1. The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Brisbane, QLD, Australia

2. Department of Veterinary and Animal Sciences, Universirty of Copenhagen, Copenhagen, Denmark

We have sampled the oral flora of a total of 103 marsupials and 5 dingoes (a total of 21 host species). The sampled animals were from a total of nine sites in the Northern Territory, Queensland, New South Wales and Tasmania. A total of 42 of the animals were wild animals (or at least wild animals temporarily in care) and the remainder were all zoo or sanctuary animals. The oral swabs were placed in transport media and were cultured within 24 hours. The swabs were plated onto 5% sheep blood agar, MacConkey agar, Mannitol Salt Agar (MSA) and into Muller-Hinton broth containing 6.5% NaCl. The modified Muller-Hinton broth was cultured onto MSA after overnight incubation at 37C. Suspect *Pasteurellaceae* were identified by colony morphology. Isolates that were catalase positive and Gram negative were regarded as "possible" *Pasteurellaceae*. Isolates that grew with typical morphology on MSA were regarded as "possible" *Staphylococcaceae*. All suspect isolates were then identified (if possible) by MALDI-TOF. A total of 427 isolates were obtained with 16 being confirmed *Pasteurellaceae* and 135 confirmed *Staphylococcaceae*. The largest sub-set of isolates (201) were organisms that the MALDI-TOF system could not identify but which had suggested identifications (at a low score) that included members of the *Pasteurellaceae*. The confirmed identifications included *Pasteurella multocida* from Tasmanian Devils, Eastern Quolls and Norhtern Quolls. *Staphylococcus aureus* was obtained from a range of animals – Tasmanian Devil, Koala, Red Kangaroo, Grey Kangaroo, Red-necked Wallaby, Northern Quoll, Eastern Quoll, Spotted-tailed Quoll, Brushtail Possum and Ringtailed Possum. Overall, the members of the family *Pasteurellaceae* and *Staphylococcaceae* are common members of the oral flora of marsupials and dingoes. The failure of the MALDI-TOF to confidently identify a large set of apparent members of the *Pasteurellaceae* suggests the possibility of novel taxa, although the data-base library lacks many of the currently recognised members of the *Pasteurellaceae*.

219

Whole genome sequencing as an improved means of identifying *Neisseria gonorrhoeae* treatment failures

Cameron Buckley[1], Brian M Forde[3, 2, 4], Tiffany Hogan[5], Monica M Lahra[5, 6], David M Whiley[7, 2, 1], Scott A Beatson[3, 2, 4]

1. Faculty of Medicine, UQ Centre for Clinical Research, The University of Queensland, Herston, QLD, Australia

2. Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, QLD

3. School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD

4. Australian Centre for Ecogenomics, The University of Queensland, Brisbane, QLD

5. WHO Collaborating Centre for STD, Microbiology Department, South Eastern Area Laboratory Services, Prince of Wales Hospital, Sydney, NSW

6. School of Medical Sciences, The University of New South Wales, Sydney, NSW

7. Pathology Queensland, Microbiology Department, Herston, QLD

Ceftriaxone is the mainstay of treatment as part of a dual therapy approach for treating gonorrhoea, however, treatment failures associated with ceftriaxone have been reported. The World Health Organisation (WHO) recommend the development of standardised protocols to verify gonorrhoea treatment failures. Currently, *N. gonorrhoeae* multi-antigen sequence typing (NG-MAST) which targets two highly variable regions (*porB* and *tbpB*), has been used extensively to examine these cases. However, these regions may mutate during therapy, inappropriately distinguishing isolates of the same strain. Two cases from Australia were previously investigated using NG-MAST; isolates from Case 1 were indistinguishable; whereas Case 2 isolates were distinguished based on an 18bp deletion in *porB*. The patient from Case 2 denied sexual contact in the follow-up period, raising questions over reliability of the NG-MAST results. Here we used whole genome sequencing (WGS) to reinvestigate Cases 1 and 2 above, with a view to examine WGS for assessing treatment failures.

Both pre- and post-treatment isolates for each Case underwent short-read Illumina sequencing, and the two post-treatment isolates underwent additional long-read PacBio sequencing. Sequence data was interrogated to determine SNP, indel and structural variation differences between pre- and post-treatment isolates.

WGS did not identify differences for isolates in Case 1. WGS confirmed the 18bp deletion for Case 2, but otherwise isolates were identical. Isolates from each Case displayed a non-mosaic *penA*, a single nucleotide (A) deletion in the *mtrR* promoter and key alterations in *porB* (G120K and A121D).

Case 2 highlights the benefits of WGS and its enhanced resolution over NG-MAST which only targets two highly variable genes. Sequencing both *porB* and *tbpB* via traditional Sanger sequencing costs approximately AU$40, whereas Illumina WGS currently costs approximately AU$100 per isolate. Although PacBio can resolve complex regions, the current cost for PacBio sequencing may prohibit its routine use for verifying treatment failures.

The WHO recommends standardised protocols for verifying *N. gonorrhoeae* treatment failures. Here, we highlight the immediate impact that WGS can have in an important and direct clinical application for *N. gonorrhoeae*. Assessing the whole genome compared to two highly variable regions provides a more confident predictor for determining treatment failure and facilitates rapid comparisons of these cases in the future.

220

In silico characterisation of the two-component system regulators of Streptococcus pyogenes

Sean J Buckley[1], David J Mcmillan[1], Peter Timms[1], Mark Davies[2]

1. Inflammation and Healing Research Cluster, University of the Sunshine Coast, Sippy Downs, Queensland, Australia

2. Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne,, Melbourne, Victoria, Australia

Bacteria respond to environmental changes through the co-ordinated regulation of gene expression, often mediated by two-component regulatory systems (TCS). Group A Streptococcus (GAS), a bacterium which infects multiple human body sites and causes multiple diseases, possesses up to 14 TCS. In this study we examined genetic variation in the coding sequences and non-coding DNA upstream of these TCS as a method for evaluating relationships between different GAS emm-types, and potential associations with GAS disease. Twelve of the 14 TCS were present in 90% of the genomes examined. The length of the intergenic regions (IGRs) upstream of TCS coding regions varied from 39 to 345 nucleotides, with an average nucleotide diversity of 0.0064. Overall, IGR allelic variation was generally conserved with an emm-type. Subsequent phylogenetic analysis of concatenated sequences based on all TCS IGR sequences grouped genomes of the same emm-type together. However grouping with emm-pattern and emm-cluster-types was much weaker, suggesting epidemiological and functional properties associated with the latter are not due to evolutionary relatedness of emm-types. All emm5, emm6 and most of the emm18 genomes, all historically considered rheumatogenic emm-types clustered together, suggesting a shared evolutionary history. However emm1, emm3 and several emm18 genomes did not cluster within this group. These latter emm18 isolates were epidemiologically distinct from other emm18 genomes in study, providing evidence for local variation. emm-types associated with invasive disease or nephritogenicity also did not cluster together. Considering the TCS coding sequences (cds), correlation with emm-type was weaker than for the IGRs, and no strong correlation with disease was observed. Deletion of the malate transporter, maeP, was identified that serves as a putative marker for the emm89.0 subtype, which has been implicated in invasive outbreaks. A bimodal motif was identified in the putative receiver domain of the Spy1556 response regulator that correlated with throat-associated A-C strains.

221

Small RNA-mediated regulation in *Acinetobacter baumannii*

Michael A Burch[1], Ralf B Schittenhelm[2], David Powell[3], Marina Harper[1], Anton Y Peleg[1, 4], Deanna Deveson Lucas[1], John D Boyce[1]

1. Infection and Immunity Program, Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Victoria, Australia

2. Monash Biomedical Proteomics Facility, Biomedicine Discovery Institute and Department of Biochemistry & Molecular Biology, Monash University, Clayton, Victoria, Australia

3. Monash Bioinformatics Platform, Monash University, Clayton, Victoria, Australia

4. Department of Infectious Diseases, The Alfred Hospital and Central Clinical School, Monash University, Melbourne, Victoria, Australia

The Gram-negative bacterium *Acinetobacter baumannii* causes life-threatening nosocomial infections, and has a near unparalleled capacity to develop multidrug-resistance. Small non-coding RNAs (sRNAs) regulate bacterial physiology, antibiotic resistance, and virulence in many pathogens; however, there has been limited characterisation of these important regulatory molecules in *A. baumannii*. Bioinformatic analysis of multiple whole-transcriptome RNA-seq datasets identified more than 40 putative sRNAs that were highly conserved in strains AB5075, AB307-0294, ATCC 17978, and ATCC 19606; four of these sRNAs were selected for initial functional characterisation. Fluorescent primer extension analyses defined the transcriptional start sites of sRNA_2, sRNA_4, sRNA_61, and sRNA_74 in strain AB307-0294, strongly suggesting they were true sRNAs. Deletion mutants in each of the four sRNA genes were constructed and analysed *via* label-free quantitative proteomics to identify protein production changes compared to the wild-type AB307-0294 strain. Notably, all four sRNA mutants showed increased production of proteins in the same fimbrial biogenesis system, which is predicted to be involved in *A. baumannii* attachment and/or biofilm formation. In addition, the *sRNA_2* mutant showed decreased production of proteins involved in D-amino acid metabolism, the *sRNA_4* mutant showed decreased production of proteins involved in type IV pili assembly and function, and the *sRNA_61* mutant showed increased production of a fibronectin-binding protein, which is predicted to be a key bacterial adhesin. Each of the sRNA mutants showed no significant change in growth or swarming motility, but the *sRNA_4* mutant showed a statistically significant, although slight, reduction in biofilm formation. In conclusion, this study has shown that *A. baumannii* sRNAs may regulate key virulence-associated proteins, and thus sRNAs may be novel candidates for therapeutic targeting in strains of multidrug-resistant *A. baumannii*.

222

Molecular epidemiology of *Mycobacterium abscessus* complex in NSW

Andrea Bustamante[1, 2], Vitali Sintchenko[1, 3], Elena Martinez[1]

1. Centre for Infectious Disease and Microbiology -Public Health, Institute of Clinical Pathology and Medical Research, Westmead, New South Wales, Australia

2. Sydney Medical School, The University of Sydney, Camperdown, NSW, Australia

3. NSW Mycobacterium Reference Laboratory, ICPMR, NSW Health Pathology- Westmead Hospital, Westmead, NSW, Australia

Mycobacterium abscessus complex (Mabc) includes three clinical relevant subspecies; *M.abscessus* subsp. *abscessus* (M.abA), *M.abscessus* subsp. *bolletti* (M.abB), *M.abscessus* subsp. *massiliense* (M.abM). It is a rapid growing mycobacterium that is distributed ubiquitously in the environment, soil and water. There has been an increase awareness of Mabc due to its emerging pathogenicity nature and drug resistant patterns globally.

Mabc is a pathogen responsible in causing infection to skin, soft-tissue and highly associated with chronic pulmonary disease, especially in those affected by Cystic Fibrosis (CF). Currently, in NSW Mycobacterium abscessus isn't a notifiable disease, therefore no epidemiology surveillance data is available that could assist in examining temporal trends of infection, drug resistance or potential outbreaks of this pathogen.

The use of Whole Genome Sequencing, can aid with the speciation of Mabc and determine markers for epidemiology clusters, drug resistance to macrolides, amikacin, ciprofloxacin and also assist in determining common markers associated with CF patient.

To date 120 Mabc positive culture isolates that were forwarded to the NSW Mycobacterium Reference Laboratory (MRL) from January 2015- December 2017. They have been whole genome sequenced by Illumina NextSeq500. On average the sequencing data had a coverage depth >100x and were assembled and analysed by recommended bioinformatics pipelines and CLC workbench. Core-genome analysis resulted in 93 M.abA with two dominant clads, 25 M.abM and 2 M.abB species. A cluster showing high genomic relatedness with < 20 Nucleotide Difference (SNPs) was observed. Common reported genotypic mutations in erm(41), rrs and rrl gene which are associated to macrolide and aminoglycoside resistance were detected among these samples and a few were confirmed by phenotypic drug susceptibility results.

223

UPEC Pathway to Chronicity: Bacterial Strain and Host Differences Determine Chronic Outcomes

Michelle N Chamoun[1], Matthew J Sullivan[1], Deepak S Ipe[1], Mark A Schembri[2, 3], Glen C Ulett[1]

1. School of Medical Science, Menzies health institute Queensland, Griffith Univerisity, Southport, Queensland, Australia

2. School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, Queensland, Australia

3. Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, Queensland, Australia

Uropathogenic Escherichia coli (UPEC) cause 80-90% of Urinary tract infections (UTI), affecting >50% of women [1]; ~25% of cases chronically recur [2]. Mouse models of recurrent UTI (rUTI), including a superinfection model, reveal a binary outcome of chronic infection: persistence, or resolution [3-5]. Recently, Interleukin-17 (IL-17) was identified as an important cytokine for the control of cystitis [6]. Here, we examined a superinfection model of rUTI using Balb/C mice to elucidate the pathway to chronicity in more detail using multidrug-resistant EC958 and urosepsis-derived CFT073 UPEC strains.

Superinfections of wild-type and IL-17-/- Balb/C mice were performed using two transurethral challenges comprising 107-108 fimbriae-enriched EC958 or CFT073 24h apart. Bladder, kidney, and urine UPEC loads were enumerated for 28 days. Chronicity was defined as ≥104 colony forming units (CFU)/ml bacteriuria at every time point [3, 4]. Multiplex cytokine assays were used to analyse plasma cytokine levels during the acute phase of infection. Enzyme-linked immunosorbent assays were used to quantitate bladder levels of IL-17 at the chronic phase of infection. Immunohistochemistry staining of sectioned kidneys was completed to identify infiltrating cell types.

EC958 had a higher prevalence of chronicity compared to CFT073 in wild-type mice, and 108 CFU infections were consistently associated with chronicity. CFT073-infected wild-type mice exhibited significantly lower bacterial loads compared to IL-17-/- mice, and IL-17 bladder levels positively correlated to bacterial load. Several inflammatory cytokines were upregulated at 24h post-infection in EC958-infected wild-type mice that progressed to chronicity, including Interleukin-6, keratinocyte cytokine, and granulocyte-colony stimulating factor, consistent with previous studies [3, 4]. Immunohistochemistry results reveal mixed inflammatory cell infiltrate in wild-type and IL-17-/- infected kidneys.

The production of IL-17 enables a conserved mechanism of immunity to control chronic UPEC rUTI. Strain and dose-related differences in the pathogenesis of rUTI in immune-competent hosts, as established in this study, define a novel role of UPEC virulence in mediating chronic rUTI.

1. Foxman, B., et al., Urinary tract infection: self-reported incidence and associated costs. Annals of Epidemiology, 2000. 10(8): p. 509-15.
2. Foxman, B., The epidemiology of urinary tract infection. Nature Reviews. Urology, 2010. 7(12): p. 653-60.
3. Hannan, T.J., et al., Early severe inflammatory responses to uropathogenic E. coli predispose to chronic and recurrent urinary tract infection. PLoS Pathogens, 2010. 6(8): p. e1001042.
4. Schwartz, D.J., et al., Uropathogenic Escherichia coli Superinfection Enhances the Severity of Mouse Bladder Infection. PLoS Pathogens, 2015. 11(1): p. e1004599
5. O'Brien, V.P., et al., A mucosal imprint left by prior Escherichia coli bladder infection sensitizes to recurrent disease. Nat Microbiol, 2016. 2: p. 16196.
6. Sivick, K.E., et al., The innate immune response to uropathogenic Escherichia coli involves IL-17A in a murine model of urinary tract infection. Journal of Immunology, 2010. 184(4): p. 2065-75.

224

Microbiological evaluation of the DEKO-190 Washer/Disinfector's ability to remove *Clostridium difficile* spores from bedpan surfaces

Deirdre A Collins[1, 2], Thomas V Riley[1, 3, 2, 4]

1. School of Pathology and Laboratory Medicine, University of Western Australia, Crawley, WA, Australia

2. School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

3. Department of Microbiology, PathWest Laboratory Medicine (WA), Nedlands, WA, Australia

4. School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia

Background and aims

Clostridium difficile is a major nosocomial pathogen that causes disease varying from mild diarrhoea to life-threatening pseudomembranous colitis. *C. difficile* spores are particularly resistant to disinfectants and frequently contaminate hospital equipment, including bedpans. Washer/Disinfectors (WDs) are commonly used to clean and decontaminate soiled equipment in health care facilities. This study aimed to evaluate the effectiveness of the DEKO-190 WD in removing *C. difficile* spores from contaminated bedpans.

Methods

Plastic carriers were inoculated with 0.2ml of a suspension of sterile human faeces containing ³ 1×10^7 CFU/ml non-toxigenic *C. difficile* spores and then taped to a sterile plastic bedpan. The bedpan and sterile negative controls were subjected to short, long or intensive wash cycles in the WD using one of two test detergents : Formula A (generic) and Formula B (highly alkaline). Counts of any residual spores on carriers were determined and mean $\log_{10}$ reductions in spores were calculated for each wash cycle.

Results

Mean $\log_{10}$ reductions were 3.21(SEM ±0.20) and 2.82 (±0.13) for Formula A and B, respectively, for the short cycle. The mean $\log_{10}$ reductions using the long wash cycle were 3.65 (±0.44) using Formula A, and 5.30 (±0.43) using Formula B, while $\log_{10}$ reductions were 3.37 (±0.58) (Formula A) and 4.64 (±0.47) (Formula B) for the intensive cycle. Residual spores were isolated from negative control carriers placed inside the machine for every wash cycle. Residual CFU titres were lower for the intensive wash program compared with the long program.

Conclusions

In conclusion, washing with the DEKO-190 significantly reduced spore concentrations on carrier surfaces on a bedpan. However, the temperature inside the DEKO-190 was not sufficient to inactivate all spores, which exhibited high temperature resistance in other heat tests. Spore counts were most effectively reduced when carriers were washed on a long or intensive wash cycle using an alkaline detergent.

225

Pseudomonas aeruginosa Trent and zinc homeostasis

Corey B. Davies[1], Mark D. Harrison[2], Flavia Huygens[1]

1. Queensland University Of Technology, Herston, QLD, Australia

2. Queensland University of Technology, Brisbane, QLD, Australia

Pseudomonas aeruginosa is a Gram-negative pathogen and the major cause of mortality in patients with cystic fibrosis. The mechanisms that *P. aeruginosa* strains use to regulate intracellular zinc have an effect on infection, antibiotic resistance and the propensity to form biofilms. However, zinc homeostasis in *P. aeruginosa* strains of variable infectivity has not been compared. In this study, zinc homeostasis in *P. aeruginosa* Trent, a highly infectious clinical strain, was compared to that of a laboratory *P. aeruginosa* strain, ATCC27853. Trent was able to tolerate higher concentrations of additional zinc in rich media than ATCC27853. Further, pre-adaptation to additional zinc enhanced the growth of Trent at non-inhibitory concentrations but the impact of pre-adaption on the growth of ATCC27853 under the same conditions was minimal. The results establish clear differences in zinc-induced responses in Trent and ATCC27853, and how zinc homeostasis can be a promising target for the development of novel antimicrobial strategies for P. aeruginosa infection in cystic fibrosis patients.

226

Diversity of carbapenem resistance bacteria and molecular identification of carbapenem resistance in marine environment

Dewa Rasmika Dewi[1], Torsten Thomas[1]

1. Centre for Marine Bio-Innovation, School of Biological, Environmental and Earth Sciences and School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia, University of New South Wales, Sydney, NSW, Australia

Carbapenem resistance bacteria (CRB) are not only restricted to clinical settings, where they cause serious infection, but can also spread within natural ecosystem. Studies on the presence of carbapenem resistance in the environment have been mostly conducted on freshwater habitats, such as rivers and lakes, and nothing known for marine ecosystem. Here, we investigated carbapenem resistance in coastal seawater along the eastern coast of Sydney. To identify potential sources for resistance, we also analysed wastewater, storm water, and terrestrial run-off water. CRBs were isolated on a range of media and identified by 16S rRNA gene sequencing. The community profile of CRBs in the different samples type were compared using qualitative unweighted UniFrac (UW) distance analysis.

A wide range of CRBs were found in the seawater samples, which differed between the locations we sampled. We found many CRBs bacteria that belonged to genera that have not been previously described to contain carbapenem resistance, including *Brevundimonas, Rheinheimera, Frondibacter, and Alteromonas*. CRBs profile in stormwater and run-off water were not different to the ones in seawater (UW sig = 0.172), indicating that they are potential sources for the CRBs found in seawater. This is also consistent with the fact that a storm event during our study had a significant impact on the CRBs profile in seawater. A genomic library for the CRBs on the genus *Rheinheimera* in *E. coli*, identified several clones that contained a gene for a novel protein related to subclass B3 metallo-beta-lactamases. As this novel gene was able to be expressed in *E. coli* and was phylogenetically related to a sequence found in *Enterobacter cloacae*, it has possibly been subject to horizontal gene transfer between *Enterbacteriacaea* and environmentally derived *Rheinheimera* (family *Chromatiaceae*).

Our study revealed that CRBs with a wide taxonomic diversity exist in marine environment, that some of them are derived from land run-off and that their carbapenem resistance genes might potentially be shared with enteric bacteria.

Poster Abstracts

227

Response of Antarctic psychrotrophic bacteria to antibiotics before and after exposure to heavy metals.

Divya Dharshini[1], Azleen Binti Mohammad Abdul Aziz [1], Chan Wai Yew[1], Fabian Amalraj[1, 2], Wan Loy Chu [1, 2], Peter Convey[3]

1. International Medical University, Bukit Jalil, Wilayah Persekutuan Kuala Lumpur, Malaysia

2. National Antarctic Research Centre, University of Malaya, Petaling Jaya, Selangor, Malaysia

3. British Antarctic Survey, Cambridge, UK

The presumption of the south polar continent, Antarctic, being pristine have posed a serious questioning in response to the recent climatic changes and disintegration of ice shelves into the ocean. Antarctica, claimed to be in the pre-antibiotic era, is uncertain due to the emergence of heavy metal resistant bacteria conferring antibiotic resistance on self-transmissible plasmids via horizontal gene transfer (HGT). Human impaction has not left this arid continent and the ecological distribution of psychrotrophs have elicited various defence mechanism in response to the myriad of stresses. Evolution of buttress microbes has introduced bacterial recalcitrance towards antimicrobial and heavy metal activity. In this study, Antarctic soil bacteria were sampled at two distinct locations- Gourlay Peninsula and Berntsen Point. Selected heavy metal (Lead, Copper and Zinc) resistant bacterial strains with minimum inhibitory concentration (MIC) ranging from 3.0 mM to 20.0 mM were isolated. Evidently, Pb and Zn resistant strains tolerated the highest concentration at 20.0 mM whereas Cu resistant strains at 13.0 mM. The most adapted bacterial strain from each heavy metal was tested with 13 different commercially available antibiotics. Prominent results were observed in isolates resistant to sulphonamides and aminoglycosides classes with a moderate incidence of co-resistance. The growth curve, biochemical characteristics, and cell surface hydrophobicity properties were attained and notable differences in the utilization of oxidase, catalase and bacterial cell adhesion by bacteria were observed post the heavy metal treatment. Promising strains will be subjected to microarray analysis for identification and gene expression profiling. These preliminary results reiterate that the Antarctic may not be exempt from the by-products of anthropogenic influences.

Keywords: Antibiotic resistance, heavy metal resistance, psychrotrophic bacteria. Antarctic soil.

228

Dsb enzymes: promising anti-virulence drug targets for Gram-negative pathogens

Rabeb Dhouib[1], Dimitrios Vagenas[1], Jason Paxman[2], Tony Wang[2], Pooja Sharma[4, 3], Jennifer Martin[5, 6], Martin Scanlon[4], Begoña Heras[2], Makrina Totsika[1]

1. Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Faculty of Health, Queensland University of Technology, Brisbane, QLD, Australia

2. Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC, Australia

3. Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts, USA

4. Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC, Australia

5. Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

6. Griffith Institute for Drug Discovery, Griffith University, Brisbane, Queensland, Australia

The resistance of various bacterial pathogens to different antibiotics constitutes a public health crisis worldwide. Due to the paucity in new antibiotic discovery and development, the need for new antimicrobials with new mechanisms of action is urgent. Developing compounds that block bacterial virulence (antivirulence) is a promising approach currently under intensive research. In Gram negative pathogens, the disulfide bond (Dsb) proteins are major facilitators of virulence; therefore, they play a key role in bacterial pathogenesis and constitute attractive targets for antivirulence drug development. Here we investigated whether our recently identified inhibitors of *Escherichia coli* K-12 DsbA can inhibit the diverse DsbA enzymes found in an important human pathogen, *Salmonella enterica*serovar Typhimurium, and attenuate its virulence. This pathogen encodes in addition to the prototypical *E. coli* K-12 DsbAB system an accessory DsbL/DsbI enzyme pair and a plasmid encoded DsbA homologue termed SrgA. Our DsbA inhibitors from two chemical classes (phenylthiophene and phenoxyphenyl derivatives) were able to block the virulence of *dsbA* null mutants complemented with structurally diverse DsbL and SrgA, suggesting that those compounds were not selective for prototypical DsbA. The ability of all compounds to inhibit DsbL was also confirmed as the activity of the native substrate of this enzyme (AssT) has decreased in the presence of the inhibitors. Modelling of DsbL- and SrgA-inhibitor interactions showed that these accessory enzymes could accommodate the inhibitors in their different hydrophobic grooves, supporting our *in vivo* findings. Further, we investigated growth of *S*. Typhimurium in the presence of DsbA inhibitors in a minimal medium. Our findings showed that some DsbA homologues are involved in growth under physiologically relevant conditions. This was confirmed by growth of isogenic *S*. Typhimurium *dsb* deletion mutants. Taken together, this work demonstrates that DsbA inhibitors can be developed to target diverse homologues found in pathogenic bacteria and this might have a fitness cost to the pathogen during infection.

229

The major evolutionary split between elasmobranchs and teleost fishes extends to the diversity partitioning of the skin microbiomes

Michael P Doane[1], Bhavya Papudeshi[1], Dnyanada Pande[1], Dovi Kacev[2], John M Haggerty[1], Megan Morris[1], Andy Nosal[3], Kevin Hovel[1], Julia Ledbetter[1], Deni Ramirez[4], Elizabeth A Dinsdale[1]

1. San Diego State University, San Diego, CALIFORNIA, United States

2. National Oceanograhic and Atmosphere Administration, San Diego, CA, United States

3. Scripps Institute of Oceanograpy, San Diego, California, USA

4. Whale Shark Mexico, La Paz, Mexico

The vertebrate lineage diverged into chondrichthyes (sharks, rays, and chimeras) and osteichthyes fishes approximately 420 mya, accumulating vast differences in their anatomy including the skin surface structure. The skin surface is the largest organ of the body, protecting eukaryotes from the external environment, and supporting diverse microscopic organisms. The fundamental processes which govern the diversity of the skin surface microbiome is expected to vary across the two lineages of fish, but this remains an outstanding question in science. Here we use whole shotgun metagenomics to test the phylosymbotic theory in the marine environment and describe the partitioning of microbiome diversity on the skin of four elasmobranchs and five teleost fish species to determine if skin surface features influence the distribution of microbiome diversity. First, we found that taxonomic and phylogenetic composition were correlated across microbiomes (Rho = 0.79), however each was only weakly correlated with gene function composition (Phylo-Func: Rho = 0.27; Taxa-Func: Rho = 0.24). Second, we found that the taxonomic and functional composition of the microbiomes where lineage specific, however do not follow a phylosymbiotic trend. Some elasmobranch species are more similar to a fish species than other elasmobranchs. Third, we show that elasmobranchs have lower taxonomic and phylogenetic α-diversity ($p < 0.05$) of their microbiome, compared with teleost fishes, while functional diversity is maintained across lineages. Last, the partitioning of the microbial diversity differed between elasmobranchs and teleost fish. We show that most diversity with each community dimension is found across individual replicates for both lineages, however sharks have more species-specific diversity than teleost. For gene functions, shark species switching, sharing more diversity than teleost fishes. Our results suggest elasmobranchs have a strong selective influence on the gene function diversity relative to teleost fishes. The findings of lower α taxonomic and phylogenetic diversity and greater gene function similarities among elasmobranchs relative to teleost fishes suggest that a lower subset of microbial species can reside at the skin interface microbiome, with specific functions necessary to reside in this niche. Our results support the hypothesis that shark skin is a unique surface where differing fundamental assemblage rules are interacting to shape the microbiome present.

230

Computational Model of *Campylobacter jejuni* Biofilms

Paulina A Dzianach[1, 2], Gary A Dykes[2], Ken J Forbes[3], Norval JC Strachan[1], Francisco J Pérez-Reche[1]

1. School of Natural and Computing Sciences, University of Aberdeen, Aberdeen, Aberdeenshire, Scotland

2. School of Public Health, Curtin University, Bentley, Western Australia, Australia

3. School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, Aberdeenshire, Scotland

The ability of bacteria to attach and grow on surfaces poses a significant challenge to both industry and human health. *Campylobacter jejuni,* an enteric human pathogenic species capable of forming biofilms, is well known for causing foodborne illness around the globe. The complexity of the interaction of bacteria with surfaces to form biofilms lends itself well to study using mathematical models. This study aims to develop a mathematical model to predict the environmental and biological conditions under which *C. jejuni* biofilms form. The model is a stochastic cellular automaton which was initially developed by using data on *Campylobacter jejuni* biofilms from the literature. Nutrient uptake, growth, autolysis, cell deactivation and extracellular matrix development are simulated as random processes. In parallel with microbial processes, both growth limiting nutrients and oxygen concentration fields are used to simulate the effect of availability of specific compounds on the microbial community. The developed model predicts the emergence of dense biofilm structures at high nutrient levels when oxygen levels allow growth. Conversely, fractal-like structures appear to develop whenever nutrients become limited. *C. jejuni* is a microaerophilic species and the model assumes that high oxygen levels inhibit biofilm development. However, there is a threshold value of oxygen concentration for each set of parameters at which there is a specific probability of biofilm forming. This suggests that the ability of bacteria to form a biofilm at the threshold depends on a range of random events, such as the way the biofilm matrix is arranged, or the order of subsequent events, such as cell division or cell autolysis. At present, the parameters used to run the simulations are based on reasonable assumptions only. The next stage of the project is to measure parameters of the model experimentally to obtain better predictions. It is hoped that this new approach to the study of *C. jejuni* biofilms will ultimately help control this pathogen.

231

Characterization of bacteriophages with specific for antibiotic-resistant *Salmonella* Typhimurium

Maheswaran Easwaran, Juhee Ahn

Bacteriophage has received renewed attention as an alternative treatment for antibiotic-resistant bacterial infections. This study aimed to investigate the possibility of using bacteriophages in controlling antibiotic-resistant *Salmonella* Typhimurium. The bacteriophages were characterized by phage adsorption, lytic activity, and specificity against *S.* Typhimurium KCCM 40253 (ST^{KCCM}), *S.* Typhimurium ATCC 19585 (ST^{WT}), ciprofloxacin-induced antibiotic-resistant *S.* Typhimurium ATCC 19585 (ST^{CIP}), and *S.* Typhimurium CCARM 8009 (ST^{CCARM}). The host ranges of phages, P22 and PBST-35, were narrow and specific to ST^{KCCM}. The highest rates of P22 adsorption were 78%, 76%, and 90%, respectively, against ST^{KCCM}, ST^{WT}, and ST^{CIP}, while the lowest adsorption rate was observed in ST^{CCARM} (73%). The latent period of P22 was 20 min against ST^{WT} and ST^{CIP}, having burst sizes of 250 and 514 PFU/cell. Respectively, at MOI of 1. The highest lytic activity of P22 was observed against ST^{KCCM}, ST^{WT}, and ST^{CIP} at 12 h post-infection. No noticeable lytic activity was observed against ST^{CCARM}. The results would provide useful information for better understanding the phage specificity for antibiotic-resistant pathogens.

1. Park, D.J., Drobniewski, F.A., Meyer, A. and Wilson, S.M. (2003) Use of a phage-based assay for phenotypic detection of mycobacteria directly from sputum. J Clin Microbiol, 680–688. Nat Rev Microbiol 8, 260–271.

2. Kim, J., Jo, A., Ding, T., Lee, H.Y. and Ahn, J. (2016) Assessment of altered binding specificity of bacteriophage for ciprofloxacininduced antibioticresistant Salmonella Typhimurium. Arch Microbiol 198, 521–529.

3. Jung, L.S. Ding, T. and Ahn, J. (2017) Evaluation of lytic bacteriophages for control of multidrug-resistant Salmonella Typhimurium. Ann Clin Microbiol Antimicrob, 16:66.

Poster Abstracts

232

Global phylogeography and ancient evolution of the widespread human gut virus crAssphage

Robert A Edwards, Bas E Dutilh, The crAssphage consortium

Microbiomes are vast communities of microbes and viruses that populate all natural ecosystems. Viruses have been considered the most variable component of microbiomes, as supported by virome surveys and examples of high genomic mosaicism. However, recent evidence suggests that the human gut virome is remarkably stable compared to other environments. Here we present the origin, evolution, and epidemiology of crAssphage, a widespread human gut virus. Through a global collaboratory, we obtained DNA sequences of crAssphage from over one-third of the world's countries, showing that its phylogeography is locally clustered within countries, cities, and individuals. We also found colinear crAssphage-like genomes in both Old-World and New-World primates, challenging rampant viral genomic mosaicism and suggesting that the association of crAssphage with hominids may be millions of years old. We conclude that crAssphage is a benign globetrotter virus that has co-evolved with the human lineage and an integral part of the normal human gut virome.

233

Evaluation of Short Incubation Disc Antimicrobial Susceptibility Testing

Li Ching Eng[1], Nerissa Jia Yi Leong, Yasmin Binte Ibrahim, Lily Siew Yong Ng[1], Thean Yen Tan[1]

1. Changi General Hospital, Singapore, SINGAPORE

Introduction and Aims

Bloodstream infections are one of the leading causes of mortality and morbidity in hospitals. Early availability of bacterial identification and antibiotic susceptibility results would reduce mortality, morbidity and hospital costs. The aims of this study were to evaluate the accuracy of short incubation (<10 hours) antibiotic susceptibility testing (AST) for positive blood cultures as compared to full incubation (16 - 20 hours). The performance standard of Muller Hinton (MH) and Muller Hinton with Thiazolyl Blue Tetrazolium Bromide (MH+TB) was also compared.

Materials and Methods

A total of 249 Gram-negative bacilli consisting of *Enterobacteriaceae* (n=209), *Acinetobacter baumannii* (n=21) and *Pseudomonas aeruginosa* (n=19) were tested against 18 antibiotics. Mixture of ESBL (n=31), ampC (n=10) and CRE (n=12) positive strains were also tested. Following inoculation of agar plates using a 0.5 Mcfarland suspension, disc zone sizes were measured by an automated disc reader after 8 hours (*Enterobacteriaceae*), 10 hours (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*), and full incubation (16 -20 hours). For each time-point, the categorical susceptibility (S, I, R) for each antibiotic was interpreted using CLSI breakpoints.

Results and Discussions

MH showed an overall average concordance rate of 92.1% compared to MH+TB (90.9%) for 13,356 antibiotic-organism comparisons. The percentage of minor errors, major errors and very major errors for MH vs MH-TB were 6.20% vs 5.90%, 0.5% vs 1.3% and 0.60% vs 0.85% respectively. Ampicillin-sulbactam, trimethoprim-sulfamethoxazole, cephalothin and cefuroxime have the lowest categorical concordance rates (<90%). This implied that the reporting of these 4 antibiotics for short incubation should be cautious. On the other hand, amikacin and meropenem have the highest categorical concordance rates (>95%).

Conclusion

Short incubation AST proved to be reliable for testing *Enterobacteriaceae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. MH demonstrated slightly better categorical concordance as compared to MH+TB. As MH is readily available commercially, it is useful for short incubation reporting. This study showed that the reporting of short incubation antibiotic susceptibility test results is highly feasible.

234

Detection and Surveillance of VISA and hVISA strains

Li Ching Eng[1], Nam Tou Choong[2], Serene Ming En Chong[2], Lily Siew Yong Ng[1], Thean Yen Tan[1]

1. Changi General Hospital, Singapore, SINGAPORE

2. Ngee Ann Polytechnic, Singapore

Introduction and Aims

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been an increasing problem in hospitals resulting high mortality. Vancomycin (VA) was the first line of treatment. In recent years, there has been an increasing incidence of reduced VA susceptibility, resulting in vancomycin intermediate (VISA) and heterogenous intermediate resistance (hVISA). The aims of this study were to compare the effectiveness of E-test and Vitek in detection of reduced VA susceptibility, performance of VA E-test on 3 Muller Hinton (MH) brands and prevalence of hVISA.

Methodology

VA susceptibility of 50 MRSA isolates, as confirmed by cefoxitin disc, was determined by VA E-test, Vitek (P-580 AST card) and microbroth dilution (gold standard). The same 0.5 McFarland inoculum was used to perform VA E-test using MH from three different manufacturers, namely BBL, Oxoid and BMX. Detection of hVISA was performed using E-test macromethod using VA and teicoplanin (TP) E-test on brain heart infusion (BHI) agar at Mcfarland 2.0.

Results

Reduced VA susceptibility (MIC = 4 mg/L) were detected in 5 out of the 50 MRSA isolates by microbroth dilution. The number of MRSA isolates with MIC > 2 mg/L as detected by E-test was 10 for BBL and 8 for both Oxoid and BMX MH plates. No MRSA isolates has been detected by Vitek to have MIC > 2 mg/L. The sensitivity for BBL, Oxoid and BMX MH for VA E-test was 100% and specificity for VA E-test was 88.8% for BBL and 93.33% for both Oxoid and BMX. The essential agreement for Oxoid and BMX was 100% (n = 50) and for BBL was 98% (n = 49). No hVISA was detected.

Conclusion

VA E-test has been proven to be more reliable than Vitek in detection of reduced VA susceptibility. Both Oxoid and BMX MH were found to perform better for VA E-test than BBL. Reduced VA susceptibility was found to be not associated with hVISA.

235

Meningococcal PorA-Loop4 induces G1 cell cycle arrest through the Akt signaling pathway

Rininta Firdaus[1, 2], Neil J. Oldfield[1], Karl G. Wooldridge[1]

1. School of Life Sciences, University of Nottingham, Nottingham, NOTTINGHAMSHIRE, United Kingdom

2. Faculty of Pharmacy, Pancasila University, South Jakarta, DKI Jakarta, Indonesia

Neisseria meningitidis (meningococcus) is a major meningitis-causing bacteria and is known for its ability to breach the blood-brain barrier (BBB). Meningococcus binds to the Laminin receptor (LAMR) on the surface of the endothelium, which is part of the blood brain barrier [1]. The meningococcal surface proteins PorA and PilQ were previously identified as the bacterial ligands responsible for binding and, subsequently the LAMR-binding moiety of PorA was localised to its fourth extracellular loop (PorA-Loop4) [2]. Using a circularised peptide corresponding to PorA-Loop 4 from *N. meningitidis* MC58, the PorA-LAMR interaction induced specific cellular responses in human brain microvascular endothelial cells (HBMECs) including G1 cell cycle arrest. Flow cytometric analysis indicated that the treatment of HBMECs with PorA-Loop4 for 24 h caused a significant reduction of cells (20%) at S-phase and a corresponding increase (23%) in the G1 population. Immunoblotting and quantitative real time PCR (qRT-PCR) analysis suggested that a blockade in Akt signaling (key proteins including Akt, GSK-3β, CyclinD1, and CDK4) contributes to the G1 arrest. Immunoblotting showed that the expression of phosphorylated GSK-3β and CDK4 were significantly increased in treated HBMECs. In contrast, the expression of phosphorylated Akt and Cyclin D1 were decreased following treatment. Transcriptome analysis using qRT-PCR confirmed that treatment of HBMECs with PorA-Loop4 peptide for 2, 4, 8, or 24 h increased gene expression of CDK4, and decreased expression of Cyclin D1. These data suggest that PorA-Loop4 induced G1 arrest through the Akt signaling pathway via Akt/GSK-3β/CyclinD1/CDK4.

1. [1]. ORIHUELA, C. J., MAHDAVI, J., THORNTON, J., MANN, B., WOOLDRIDGE, K. G., ABOUSEADA, N., OLDFIELD, N. J., SELF, T., ALA'ALDEEN, D. A. & TUOMANEN, E. I. 2009. Laminin receptor initiates bacterial contact with the blood brain barrier in experimental meningitis models. J Clin Invest, 119, 1638-46.
2. [2]. ABOUSEADA, N. M., ASSAFI, M. S., MAHDAVI, J., OLDFIELD, N. J., WHELDON, L. M., WOOLDRIDGE, K. G. & ALA'ALDEEN, D. A. 2012. Mapping the laminin receptor binding domains of Neisseria meningitidis PorA and Haemophilus influenzae OmpP2. PLoS One, 7, e46233.

236

Variation in parvovirus B19 IgG seroprevalence between different states in Australia

Robert L Flower[1], Elise C Gorman[1], Veronica C Hoad[1], Francesca D Frentiu[2], Sarah Tozer[3], Seweryn Bialasiewicz[3], Helen M Faddy[1]

1. Australian Red Cross Blood Service, KELVIN GROVE, QLD, Australia

2. School of Biomedical Sciences and Institute for Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland, Australia

3. Queensland Paediatric Infectious Diseases Laboratory, Centre for Children's Health Research, Brisbane, Queensland, Australia

Parvovirus B19 (B19V) is a DNA virus with a global reach. Infection results in a variety of clinical presentations, including erythema infectiosum in children and arthralgia in adults. Transmission is usually through the respiratory route, however, vertical transmission, transfusion-transmission, as well as transmission through solid organ or haematopoietic transplantation, have been documented. There is limited understanding of current seroprevalence of antibodies to B19V in the Australian population. This study aimed to provide a current estimate of B19V IgG seroprevalence in a cohort of Australian blood donors and in a pediatric population. Age/sex/region stratified plasma samples (n=2,221) were collected from Australian whole blood donors. Samples were also sourced from pediatric patients (n=223) in Queensland. All samples were screened for B19V IgG using indirect-based enzyme-linked immunosorbent assay. Overall 57.90% (95% CI: 55-94-59.85) of samples tested positive for B19V IgG, indicating prior exposure to this virus. The national age-standardized seroprevalence of B19V IgG in Australian's aged 0 to 79 years was estimated to be 54.41% (95% CI: 54.39-54.43%). Increasing age ($p<0.001$) and state of residence ($p<0.001$) were independently associated with B19V IgG seropositivity in blood donors, while sex was not ($p=0.547$). A large amount of variation in B19V IgG prevalence between the states was observed, with the highest rate observed in donors from Tasmania (71.88%, 95% CI: 66.95-76.80%) and the lowest in donors from the Northern Territory (52.56%, 95% CI: 46.84-58.28%). This study demonstrates a clear association between B19V seroprevalence and increasing age, with over half of Australians likely to be immune to B19V. Differences in seroprevalence were also observed in donors from different states, with a higher prevalence in those from the southern states, which is consistent with previous studies that show higher rates in countries with a higher latitude. This study provides insight into the seroprevalence of B19V IgG in the Australian population, which has implications for public health as well as transfusion and transplantation safety in Australia.

237

Comparison of pre-extraction depletion methods for culture-independent sequencing of *Bordetella pertussis*

Winkie Fong[1, 2], Verlaine Timms[1], Vitali Sintchenko[1, 2]

1. Centre of Infectious Diseases and Microbiology - Public Health, Westmead, NSW, Australia

2. Marie Bashir Institute, University of Sydney, Sydney, NSW, Australia

Bordetella pertussis, the main etiological agent of whooping cough, is highly infectious and despite widespread vaccination, is re-merging as a significant pathogen globally. In the pre-vaccination era, *B. pertussis* the leading case of infantile death due to an infectious disease globally. In the decades following the introduction of vaccination in Australia there were on average 5000 notifications per year, in 2011 there were over 38,000 and questions remain as to why widespread vaccination cannot control this highly infectious disease. Thus, molecular surveillance of currently circulating strains becomes paramount to the control of *B. pertussis* by providing information on strains and for future vaccine development.

However, surveillance of *B. pertussis* relies on laboratory isolates, yet since the early 1990s, *B. pertussis* is primarily detected in clinical samples by PCR. Thus, live culture isolates are rarely collected and there is now little material for molecular surveillance.

The objective was to develop a method, independent of culture, to detect and type *B. pertussis* in respiratory specimens to provide molecular surveillance data.

The study compared commercial depletions kits and specimen-processing methods using selective lysis detergents and determine which method produced optimised data yield by assessing DNA concentration and purity. Commercial depletion kits included the MolYsis Kit, Qiagen Microbiome Kit, NEBNext Kit on *B. pertussis* spiked nasopharygeal aspirates, following manufacturer protocols. Saponin was used as published previously by Hasan et al (2016). Samples were then tested by rtPCR for ERV3 and *IS481*, then sequenced and analysed.

Results showed that while commercial kits significantly reduced the human DNA within the sample, it also reduced the concentration of *B. pertussis* as well. However, selective lysis with Saponin resulted in an almost undetectable level of human DNA, with minimal loss of bacterial DNA. Read depth improved with a 500% increase in sequencing reads that belonged to *B. pertussis*. This investigation drew upon the need for new methods of surveillance of *B. pertussis* and delivered a potential protocol.

238

Food loss – the microbiology of food deterioration

Edward M Fox[1], Sean C Moore[1], Tanoj Singh[1], David Beale[2], Mary Ann Augustin[1], Scott Chandry[1]

1. CSIRO, Werribee, VIC, Australia

2. CSIRO, Dutton Park, QLD, Australia

Approximately one-third of food produced globally is not consumed but instead lost from the food supply chain. A growing global population, food shortages particularly in Third World countries together with environmental pressures such as water shortage and climate change are placing increasing strains on global food demands. The diversion of food loss back into the food supply chain could provide a significant contribution to meeting these ever increasing food demands. In order to achieve this, however, it is essential to understand the safety and stability of food loss biomass to evaluate its suitability as a new food source. In this study we selected apple pomace, a food loss biomass produced during the production of fruit juices. Apple pomace represents about 30% of the total biomass of an apple and contains the majority of the fibre and protein components. Storage trials were conducted at 4 °C and 8 °C over 7 days to determine the change in microbial load of apple pomace (bacteria, yeast and mould counts), as well as the metabolite profiles (GC-MS). Regardless of storage temperature used, the bacterial community reached similar levels after 7 days, a small reduction in fungal load was achieved at 4 °C relative to 8 °C at 7 days. Analysis of the metabolites showed that despite similar microbial loads, distinct patterns were associated with 4 °C versus 8 °C storage. At 8 °C many of the sensory compounds associated with apple taste and smell were reduced significantly, and this was coupled with a large increase in ethanol suggesting microbial fermentation. In contrast, 4 °C retained these sensory components and limited ethanol production. Taken together these results suggested small changes in storage temperatures drive differences in the microbial population and associated metabolism of stored apple pomace, which may have important consequences for its subsequent utilisation as a food ingredient.

239

Group B Streptococcus bacteriophages with broad clinical host range

Lucy L Furfaro[1], Barbara J Chang[2], Matthew S Payne[1]

1. Medical School, Division of Obstetrics and Gynaecology, The University of Western Australia, Perth, WA, Australia

2. School of Biomedical Sciences, The Marshall Centre for Infectious Diseases Research and Training, The University of Western Australia, Perth, WA, Australia

As a leading cause of sepsis in neonates, *Streptococcus agalactiae* or Group B Streptococcus (GBS) is a significant obstetric pathogen. Numerous screening strategies have been implemented world-wide to identify mothers at risk of transmitting the organism to their newborn. Risk-based strategies and detection of GBS by culture screening all result in antibiotic administration. Although penicillin remains effective, resistance is inevitable and the impact of antibiotic administration on the maternal and neonatal microbiomes is not well-understood. An ideal treatment option would be one in which GBS are targeted in colonized women, with no effect on commensal organisms. Use of bacteriophage (phage) therapy, is one such option. Four phages (LF1 – LF4) were isolated from wastewater using standard enrichment techniques. All phages showed varied and broad ranging activity against clinical GBS isolates collected from pregnant women (colonising) and neonates (disease). Phages displayed lytic activity *in vitro* against antenatal GBS isolates with 73.3% of GBS isolates (n = 135) susceptible to at least one phage. LF2 and LF4 showed activity against all neonatal disease-causing isolates (n=10), while LF1 was also active against 90% of these isolates. Transmission electron microscopy confirmed all phages as members of the *Siphoviridae* family. Whole genome sequencing revealed genomes ranging from 32,205 – 44,768 bp. LF1 and LF4 share 99.9% nucleotide identity and are closely related to putative prophage of GBS. LF2 shows homology to a different putative prophage, although its genome organisation differs, while LF3 exhibits genome similarity to a *Streptococcus pyogenes* phage. The presence of genes required for lysogeny including integrase, repressor and regulatory modules suggests all are temperate phages, which are not typically used for phage therapy, however, the *in vitro* activity of these phages against a broad range of clinically important isolates is extremely promising and they may have therapeutic use as bioengineered phages or purified lysins.

240

A preliminary comparison of five assays for detecting past exposure to *Coxiella burnetii* for use prior to human Q fever vaccination

Stephen R. Graves[1], Paul Ross[2], Chelsea Nguyen[3], Meg Jeppesen[2], John Stenos[3], Jenny Robson[2]

1. Australian Rickettsial Reference Laboratory, New Lambton, NSW, Australia

2. Sullivan Nicolaides Pathology (SNP), Brisbane

3. Australian Rickettsial Reference Laboratory, Geelong, VIC, Australia

Background

Vaccination against Q Fever (*Coxiella burnetii* infection) is performed in Australia using Q-VAX, a formalin-killed whole cell vaccine. Adverse reactions occur in those persons with prior exposure to *C.burnetii*. To prevent this adverse reaction pre-vaccination screening is performed and persons found to be positive are excluded from vaccination. Currently a skin test (to detect T-cell immunity) and an antibody assay (to detect B-cell immunity) are undertaken on each person prior to vaccination. However the assay that "best" correlates with prior exposure to *C.burnetii* and thus adverse reactions to the vaccine is not known.

A new interferon-gamma release assay (IGRS), "Q-Detect", has recently become available. This new assay and 4 current assays were compared.

Material/Methods

A small group (n=25) of attendees at an Australian scientific conference were offered Q-VAX vaccination. As part of their pre-vaccination testing, 5 different assays were performed on them to detect their possible prior exposure to *C.burnetii*. The assays were:

1. intradermal skin test inoculation
2. serology by immunofluorescence (IF)
3. serology by enzyme immunoassay (EIA)
4. serology by complement fixation test (CFT)
5. IGRA (the new assay)

Results

Of the 25 participants in the study, 7 had prior exposure to *C.burnetii* due to vaccination against Q Fever and 1 had prior Q Fever infection. The remaining 17 persons had no known prior exposure to *C.burnetii*.

The results from the 5 assays were compared in the 2 groups and the sensitivity and specificity of the assays calculated, as under:

Assay	Sensitivity	Specificity	Conclusion
1 skin test	37%	76%	Poor
2 serology IF	62%	100%	Poor
3 serology EIA	25%	100%	Poor
4 serology CFT	12%	100%	Poor
5 IGRA	100%	82%	Good

Conclusion

The new IGRA "Q-Detect" is superior to the 4 currently used assays for detecting prior exposure to *C.burnetii* in persons seeking vaccination against Q Fever in Australia.

241

Biofilm screening of *Listeria monocytogenes* from common MLST subtypes present in food production environments.

Jessica Gray[1], Scott Chandry[1], Chawalit Kocharunchitt[2], Mandeep Kaur[2], John Bowman[2], Ed Fox[1]

1. CSIRO, Werribee, VIC, Australia

2. University of Tasmania, Hobart, Tasmania

Listeria monocytogenes is a food borne pathogen capable of causing high hospitalisation and mortality rates. Populations of highest risk include the elderly, immunocompromised, pregnant women and neonates. In addition, *L. monocytogenes* is frequently identified as the causative agent responsible for microbial food recalls. Due to its ubiquitous nature, *L. monocytogenes* is a difficult microorganism to prevent from entering and colonising food production environments (FPEs). Some strains, known as persisters, may colonise the FPE and remain despite regular cleaning and sanitising regimes. These persistent strains act as a repetitive source of cross contamination and have been shown to persist over a substantial number of years. Factors influencing the persistence and survival of *L. monocytogenes* in FPEs are not well characterised; however, are postulated to be influenced by a number of genetic and environmental elements. One factor of significant influence is the bacterium's ability to adhere to surfaces and form biofilms. Biofilms are structures containing microbial cells attached to each other and/or a surface housed in an extracellular polymeric substance. Biofilms offer a number of advantages to bacterial species in FPEs such as: multiple species in various metabolic states; increased protection from disinfectants, desiccation and other environmental elements; removal of toxic metabolites; transfer of nutrients; and the opportunity to acquire new genetic traits through horizontal gene transfer. Previous studies have tried to associate a particular serotype or pulsotype to increased biofilm formation; however, differing results have been reported. This study utilised 52 isolates from various MLST subtypes commonly identified in FPEs to assess attachment and biofilm formation over 96 h under conditions reflective of the FPE. The results indicated attachment and biofilm formation may be strain specific, with no individual MLST subtype demonstrating increased ability to attach or form biofilms. This information will help to further understand how *L. monocytogenes* survives in FPEs with further research required to identify potential strain specific differences.

Poster Abstracts

242

The role of the RNA chaperone ProQ in the Gram-negative bacterium *Pasteurella multocida.*

Emily L Gulliver[1], Brandon Sy[2], Julia Wong[2], Deanna Deveson Lucas[1], Ralf B Schittenhelm[3], David Powell[4], Jai Tree[2], Marina Harper[1], John Boyce[1]

1. Infection and Immunity Program, Monash Biomedicine Discovery Institute and Department of Microbiology, Monash University, Clayton, Vic, Australia

2. School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia

3. Monash Biomedical Proteomics Facility, Monash Biomedicine Discovery Institute and Department of Biochemistry and Molecular Biology, Monash University , Clayton, Vic, Australia

4. Monash Bioinformatics Platform, Monash University, Clayton, VIC, Australia

The Gram-negative bacterium *Pasteurella multocida* is the causative agent of many diseases, including fowl cholera in chickens. One mechanism by which bacteria regulate transcript abundance and protein production is riboregulation, which involves a specific interaction between a small RNA (sRNA) and an mRNA that acts to alter transcript stability and/or translational efficiency. Hfq is a well-characterized RNA chaperone protein that is involved in bacterial riboregulation. Recently, a second RNA chaperone called ProQ was shown to play a critical role in stabilizing some sRNA/mRNA interactions. To assess the role of *P. multocida* ProQ in riboregulation, we analyzed the transcriptome of a *proQ* TargeTron® mutant using RNA-seq. In total, 35 transcripts showed increased expression and 96 showed decreased expression. Both sRNAs and tRNAs were highly overrepresented in the set of differentially regulated genes (p = 3.76 x $10^{-9}$and p = 3.78 x 10^{-11}, respectively); 17 tRNAs and eight sRNAs showed increased expression and 14 sRNAs showed decreased expression. Direct interactions between ProQ and one sRNA, five tRNAs, and 28 mRNAs involved in the production of ribosomal proteins, were confirmed using UV-crosslinking, ligation, and sequencing of hybrids (UV-CLASH). Several tRNAs formed hybrids with other tRNAs, mRNAs and sRNAs, indicating that these can bind to other RNA species while bound to ProQ. In summary, our results indicate that ProQ binds to many different RNA species, and in particular, to sRNAs and tRNAs. Moreover, transcriptomic analysis indicates that ProQ interaction can stabilize some sRNA species but can destabilize many tRNA species.

243

Evolution of a Clade of *Acinetobacter baumannii* Global Clone 1, Lineage 1 via Acquisition of the *oxa23* Carbapenem Resistance Gene and Dispersion of ISAba1

Mohammad Hamidian[2, 1], Jane Hawkey[3], Ryan Wick[3], Kathryn E Holt[3], Ruth M Hall[2]

1. University Of Technology Sydney, Ultimo, NSW, Australia

2. University Of Sydney , DARLINGTON , NSW, Australia

3. University of Melbourne, Melbourne, VIC, Australia

Acinetobacter baumannii is a gram-negative opportunistic pathogen that causes a range of hospital-acquired infections. Antibiotic resistance is a critical problem in *A. baumannii*, particularly when resistance genes are acquired by members of successful globally-distributed clones e.g. global clone 1 (GC1). Here, we investigated the evolution of an expanding sub-clade of multiply antibiotic resistant GC1 associated with carbapenem and aminoglycoside resistance.

Twenty-seven strains belonging to a specific clade of GC1 were identified, 3 in our collection and 24 in GenBank, using a range of criteria including the carriage of the Tn*6168* transposon, carrying the ISAba1-*ampC* structure, responsible for resistance to 3rd generation cephalosporins, in a specific chromosomal location, and a specific Outer Core oligoaccharide, OCL3. The genome sequence of the representative of Australian isolates, which was also resistant to carbapenems, was determined using Illumina HiSeq and PacBio long-read technology. A range of bioinformatics tools was used to examine the context of resistance genes, distribution of the chromosomal ISAba1 copies, and phylogeny.

Bayesian analysis showed that the Tn*6168*/OCL3 clade arose in the late 1990s, from an ancestor that had already acquired resistance to third generation cephalosporins and fluoroquinolones. Between 2000 and 2002, this clade further diverged into distinct sub-clades by insertion of AbaR4 (carrying the *oxa23* carbapenem resistance gene) at a specific chromosomal location in one group, and a phage genome in the other. Both subgroups show evidence of ongoing evolution of resistance loci and ISAba1 dispersal. Most concerning, this includes introduction of the *armA* aminoglycoside resistance gene via AbGRI3, acquired from a GC2 isolate.

Our analysis revealed the complexity of genetic events leading to resistance to multiple antibiotics in the Tn*6168*/OCL3 clade of GC1. Comparison of IS insertions sites with the dated phylogeny shows ISAba1 first entered this clade in around mid 90s with the cephalosporin resistance transposon Tn*6168* and has since expanded in both sub-clades. It also revealed multiple routes for the acquisition of the *oxa23* carbapenem resistance gene.

244

Detection and quantification of the heterogeneous *S. aureus* populations to identify antibiotic-induced persistence

Marwa M. Hassan[1], Mark S. Butler[1], Andrea Ranzoni[1, 2], Matthew A. Cooper[1]

1. The University of Queensland, Institute for Molecular Bioscience, Brisbane, QLD, Australia

2. Bioengineering Department, Ellume Pty Ltd, Brisbane, Queensland, Australia

Introduction

Persister cells are characterised as being viable but non-culturable, a state that preserves their metabolic energy to survive the environmental stress, which allows for recurrent infections. Detection of persisters is therefore not possible with standard culture-dependent methods. Furthermore, the effect of antibiotics on the development of persisters has not been assessed. This study aimed to identify antibiotic-induced persistence and determine the percentage of heterogeneity.

Methods

Vancomycin, daptomycin and dalbavancin were assessed by standard MIC methods against selected *Staphylococcus aureus* strains. Replicates of MIC assays were stained with propidium iodide to quantify live/dead and a reactive oxygen species (ROS) dye to detect and quantify persisters using culture-independent single-cell sorting, independently. A comparative analysis was then performed.

Results

Dalbavancin showed the lowest MIC values against tested *S. aureus* strains followed by daptomycin and vancomycin. Cell sorting of vancomycin-, daptomycin- and dalbavancin-treated *S. aureus* strains showed a range of 1.9-10.2%, 17.7-62.9% and 7.5-77.6% live cells based on the strain, respectively, in which daptomycin, in particular, was a strong inducer of a persister population. Persisters represented 3.7-16% of the bacterial population.

Conclusions

The culture-independent identification of antibiotic-induced persistence through studying at the single-cell level showed different efficacy of antibiotics than standard MIC. Vancomycin was the most effective antibiotic against tested strains followed by dalbavancin then daptomycin as assessed by cell sorting. Therefore, re-evaluation of standard MIC methods may be required to assess the efficacy of antibiotics. Additionally, the detection of daptomycin-associated persisters may provide elucidation to the reported rapid resistance development *in vivo*.

245

Optimisation of antibody effector functions in HIV neutralising monoclonal antibodies

Behnaz Heydarchi, Jack Edwards, Christopher Gonelli, Damian Purcell

Introduction: On of the most effective bio-therapeutics for HIV to date are the therapeutic monoclonal antibodies, particularly those that bind to antigen and recruit the immune effector functions simultaneously. However, antibody-based therapeutics are often only effective in a subset of patients and a significant effort is essential to fine-tuning the properties of antibodies to achieve high efficacy. Constant fragment (Fc) engineering is one of the most efficient strategies to modulate the interaction of monoclonal antibodies with humoral and cellular components of the immune system. Human Fc gamma receptors (FcγR) bind to antibody Fc region and mediates antibody effector functions such as antibody dependent phagocytosis (ADP) and antibody-dependent cell-mediated cytotoxicity (ADCC). FcγRIIa is found on macrophages, neutrophils and eosinophils and contributes in ADP whereas FcγRIIa is mainly present on NK cells and involves in ADCC. In this study we aimed to engineer the Fc region of an anti-HIV broadly neutralising antibody to optimise its binding to human FcγRs contributing in ADP and ADCC functions.

Methods: Bovine HIV broadly neutralising antibody NC-COW1 (Sok et al., 2017) was chimerised by cloning of variable heavy and light genes into the vectors expressing human heavy and light constant regions. The human IgG1 heavy constant region was mutated in an attempt to enhance the binding of the antibody to FcγRIIa and FcγRIIa. We made 4 different formats of NC-COW1 with the following mutations in Fc region: 1- No mutation; 2- S239D, I332E, A330L; 3- G236A, S239D, I332E; 4- G236A, S239D, I332E, A330L (Lazar et al., 2006; Richards et al., 2008). Format 2 and 3 were constructed to improve ADCC and ADP activity, respectively while format 4 had both enhancing mutations.

Results: Our results showed that chimeric NC-COW-1 without any optimising mutations had some interactions with FcγRIIa and FcγRIIa. However, among all different formats, the antibody with 4 mutations showed the highest binding to FcγRIIa and FcγRIIa. Further experiments will be performed to confirm the enhanced antibody effector functions in in vitro.

Conclusion: The enhancements in human Fc gamma receptor binding described here provide the potential to improve the performance of therapeutic antibodies targeting HIV-1 viruses.

246

Host-derived lactate is an important *Haemophilus influenzae* carbon source

Jennifer Hosmer, Marufa Nasreen, Horst Joachim Schirra, Alastair McEwan, Ulrike Kappler

Non-typeable *Haemophilus influenzae* (NTHi) is a human respiratory pathogen involved in upper and lower respiratory tract diseases, including COPD, cystic fibrosis, and asthma[1, 2]. Despite extensive studies of NTHi virulence, growth substrates used *in vivo* have not been unambiguously identified. Here, we investigated the role of lactate, a universal metabolite in humans, in NTHi growth. NTHi substrates were investigated using phenotypic microarrays (Omnilog), extracellular metabolomics of NTHi-infected and uninfected submerged tissue cells (TCs), and mutant phenotypic characterisation.

Metabolomic analyses revealed that, in addition to glucose, NTHi consumes TC-derived lactate, which was a highly used substrate in the microarrays. Additionally, the growth rate of Hi2019 and Hi2019 respiration on L-lactate were 1.5-fold and 13-fold greater than on D-lactate, suggesting NTHi primarily uses L-lactate.

We bioinformatically identified two Hi lactate utilisation systems; 84% of strains metabolised L- and D-lactate via membrane-bound L-lactate (LldD) and D-lactate (Dld) dehydrogenases and a cytoplasmic NAD^+-dependent D-lactate dehydrogenase (LdhA), while 16% contained the L-lactate-specific LutABC system. In Hi2019, LldD activity decreased, while Dld and LdhA activity increased, with reduced oxygen, suggesting LDHs are oxygen-regulated.

Additionally, Hi2019$^{\Delta lldD}$, Hi2019$^{\Delta dld}$, and Hi2019$^{\Delta ldhA}$ mutants had impaired L- and D-lactate respiration and, following the removal of one LDH gene, the activities of the remaining LDHs in the mutants was significantly decreased compared to the wild type control. This suggested that LldD, Dld, and LdhA activity is interdependent. Interestingly, only Hi2019$^{\Delta lldD}$ had reduced growth, by 2-fold, on lactic acid, suggesting LldD, which was highly expressed in Hi2019 TC co-cultures, is central for NTHi energy generation on lactate and is currently being investigated in co-culture. From this work, we conclude that TC-derived lactate is an important NTHi carbon source and that targeting this lactate metabolism could undermine NTHi growth during infection.

Poster Abstracts

247

The enemy of my enemy is my friend: eliminating *Legionella* in premise plumbing biofilms using bacteriophages

Casey K Huang[1], Karen Weynberg[2], Philip Bond[1]

1. Advanced Water Management Centre, The University of Queensland, Brisbane, Queensland, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

Legionella can cause human respiratory disease which includes the severe, pneumonia-like form called Legionnaires' disease (LD). Originating from natural environments such as freshwater and soil, *Legionella* can inhabit infrastructure including engineered water systems or cooling towers, from where contaminated aerosols can transmit to susceptible human populations, e.g. the vulnerable elderly and immunocompromised patients in healthcare facilities. Nosocomial infections can result in high mortality rates of up to 50%.

This is a major concern to Queensland and Australian healthcare facilities, where multiple outbreaks of the disease caused by *L. pneumophila* have occurred. In these outbreaks, the infectious source has been traced to persistent biofilms present in drinking water distribution systems (DWDS), such as the plumbing systems of hospital buildings. Despite low nutrient conditions and residual disinfectants, biofilms develop and persist in these plumbing systems. These biofilms are likely to favourably support the complex ecology, persistence and increased pathogenicity of waterborne opportunistic pathogens like *Legionella*.

This project aims to improve understanding of the biofilm ecology of *Legionella* in DWDS, as well as to determine alternative antimicrobial strategies to remove *Legionella* from persistent biofilms. One strategy is to use bacteriophages (phages) to selectively target and remove *Legionella* from biofilms. This approach has been successful in medical and industrial applications on various bacterial species. Although currently there is limited research on phages specific for *Legionella*, isolating and using synthetic biology approaches to engineer *Legionella*-specific phages with superior properties has great potential for their control.

To isolate *Legionella*-specific phages, we are targeting various water samples that contain *Legionella* as detected by PCR. Sources positive for *Legionella* spp. include water samples from buildings, cooling towers, riverbanks, creek water and wastewater. These samples have been screened for phages against various *Legionella* strains using standard phage isolation techniques. Phages specific for *Legionella* have been isolated, and these are currently undergoing characterisation with regard to host range, host infectivity, and genome sequencing.

248

Recent Laboratory Findings of *Brucella abortus* in South Korea

Seon Do Hwang[1], Kyeong Ah Lee[1], Mi Yeon Kim[1], Byung Hak Kang[1], Jae Ok Kim[1]

1. Division of Bacterial Diseases, Korea Center for Disease Control and Prevention, Cheongju-si, CHUNGCHEONGBUK-DO, South Korea

Brucellosis is caused by the genus *Brucella* and is known as a major bacterial zoonotic disease. Although there has been great progress in controlling the disease in many countries, there still remain regions where the infection persists in domestic animals and, consequently, transmission to the human frequently occurs. In Korea, the number of human brucellosis cases increased sharply to 215 in 2006 when the number of outbreaks also reached a peak among animals. The number of human cases and bovine outbreaks has declined starting in 2007 by continuous effort of eradication in bovine brucellosis. Since the first case of human brucellosis in 2002, all brucellosis cases were caused by *B. abortus* except the overseas inflow cases of *B. melitensis*. In this study we introduce three isolated human *B. abortus* cases in 2016~2017 which were requested to Korea CDC.

Suspected 55 blood samples of brucellosis were sent to Korea CDC in 2016~2017 and inoculated into media of BACTEC system to cultivate. After several passages using Brucella agar plate, the pure isolates were examined by classical biotyping methods, and PCR test using Brucella-specific primers, *Brucella* species specific multiplex PCR, and real time PCR. The sequence from PCR was also analysed.

As a result, 3 of 55 samples were identified as *B. abortus*. They were grown in Brucella agar plate and showed positive result for H_2S production, oxidase and urease. DNA from isolates were extracted and amplified in *Brucella* genes and were positive in *B. abortus* specific multiplex PCR. In real time PCR these showed positive results in *B. abortus* specific target genes.

These three cases identified as *B. abortus* had clinical history of eating uncooked meat or contacting with cattles. In Korea, *B. abortus* biotype 1 were major pathogenic species in human and animals. In these cases there is need to investigate more about biotyping and molecular typing between human isolates and cattle isolates to understand the exact relationship. Although the case of brucellosis in human and animals become low, people need to know *B. abortus* is still exist and pose a threat to human in Korea.

Acknowledgements: This work was supported by Korea Centers for Disease Control and Prevention

(2016-NG52002-00, 4837-301-210-13)

249

Atmospheric trace gas oxidation supports persistence of the environmentally abundant phylum Chloroflexi

Zahra Islam[1], Joanna Feng[1], Carlo Carere[2], Chris Greening[1]

1. School of Biological Sciences, Monash University, Clayton, VIC, Australia

2. GNS Science, Lower Hutt, New Zealand

The majority of bacteria within aerated environments exist within a variety of dormant forms (1). In this state, bacteria adapt to adverse environmental conditions such as organic carbon starvation by reducing metabolic expenditure and potentially using alternative energy sources (2,3). In this study, we investigated the energy sources that could sustain persistence of the environmentally widespread and abundant bacterial phylum Chloroflexi. We provide evidence that three strains from this phylum can persist during organic carbon limitation by scavenging trace concentrations of molecular hydrogen (H_2) and carbon monoxide (CO) found within the atmosphere. Phylogenetic analysis shows that the enzymes required for atmospheric H_2 and CO oxidation, namely the group 1h [NiFe]-hydrogenase and carbon monoxide dehydrogenase, are widely distributed in aerobic heterotrophic strains of Chloroflexi.

Studies focusing on *Thermomicrobium roseum* as a model organism showed that the operons encoding these enzymes are significantly upregulated during the transition from active growth to persistence. We validated by gas chromatography that this strain oxidises atmospheric H_2 and CO during organic carbon starvation. Moreover, we validated that the sporulating strains, *Ktedonobacter racemifer* and *Thermogemmatispora* T81, also mediate scavenge of these gases during persistence. This study demonstrates for the first time the ability of Chloroflexi, the sixth most dominant soil phylum (4), persist by using atmospheric energy sources and uncovers new sinks in the biogeochemical cycles of H_2 and CO. Our findings also suggest that trace gas oxidation is present in more bacterial phyla than previously thought.

1. RAPPÉ, M. S. & GIOVANNONI, S. J. 2003. The Uncultured Microbial Majority. Annual Review of Microbiology, 57, 369-394.
2. GREENING, C., BISWAS, A., CARERE, C. R., JACKSON, C. J., TAYLOR, M. C., STOTT, M. B., COOK, G. M. & MORALES, S. E. 2016. Genomic and metagenomic surveys of hydrogenase distribution indicate H(2) is a widely utilised energy source for microbial growth and survival. The ISME Journal, 10, 761-777.
3. SØNDERGAARD, D., PEDERSEN, C. N. S. & GREENING, C. 2016. HydDB: A web tool for hydrogenase classification and analysis. Scientific Reports, 6, 34212.
4. JANSSEN, P. H. 2006. Identifying the Dominant Soil Bacterial Taxa in Libraries of 16S rRNA and 16S rRNA Genes. Applied and Environmental Microbiology, 72, 1719-1728.

250

Evaluation of an RNA extraction control for routine laboratory-developed reverse transcriptase-PCR diagnostic tests

MUHAMMAD FAHMI ISMAIL[1], LIAT HUI LOO, MATTHIAS MAIWALD

1. KK WOMEN'S AND CHILDREN'S HOSPITAL, Singapore, SINGAPORE

Introduction
Our laboratory performs nucleic acid extraction for diagnostic PCR tests. The efficiency of the extraction process is currently not assessed. Thus, we evaluated an RNA Extraction Control 670 (REC) (Bioline Inc, USA) which can fulfil this purpose for RT-PCR tests across varying sample types.

Method
A total of 80 patient samples which were submitted for reverse transcriptase (RT)-PCR tests were from four main sample types: cerebrospinal fluid (CSF), swabs, rectal swabs or stool, and blood samples. These samples were extracted and the RT-PCR master mix was prepared according to the respective laboratory protocols. Each sample was processed in duplicate. One replicate was spiked with 2 µl REC RNA prior to the extraction process. This replicate's RT-PCR master mix tube was prepared with additional 1 µl REC control mix. All the samples were run on real-time thermocyclers with an additional detection channel at 705 nm for REC detection.

Results
REC was detected in all RT-PCR runs with varying median cycling threshold (Ct) values across different sample types. The median Ct values were 11.30 (10.45-12.61) for CSF, 21.97 (12.45-26.01) for swabs, 13.33 (12.93-20.99) for rectal/stool samples and 15.38 (14.94-17.36) for blood. Out of 80 samples tested, 10 were positive for their respective main targets and the corresponding REC-spiked reaction tubes displayed similar Ct values to the non-spiked reaction tubes, with a mean difference of 0.56 Ct values.

Discussion
The results showed that the REC control is suitable for monitoring the extraction process of all four different sample types. The difference in median Ct values in different sample types may be attributed to the different RT-PCR cycling conditions of the tests involved. Inhibition of REC amplification was not detected in any of the REC-spiked samples, suggesting that REC did not interfere with the RT-PCR detection.

251

A prostate cell line model for persistent Zika virus replication

Rhys Izuagbe[1], Daniela Loessner, Liesel Stassen[1], Francesca Frentiu[1]

1. Institute of Health and Biomedical Innovation, Queensland University of Technology, Brisbane, QLD, Australia

Infection with Zika virus (ZIKV) can lead to congenital malformations in babies and Guillain-Barré syndrome in adults. ZIKV is a RNA virus from the Flaviviridae family and is predominantly transmitted by the mosquitoes Aedes aegypti and Aedes albopictus. Uniquely, the virus can also be sexually transmitted. It can be detected in the semen of infected men for up to 6 months in some cases. Recent work aimed at understanding sexual transmission has focused on the testes as a reservoir. However, cases of ZIKV sexual transmission and persistent virus shedding from vasectomised men indicate that other reservoirs exist in the male reproductive tract. The prostate is a strong candidate as a ZIKV niche, given its high secretory function and contribution to ejaculate. Here, we develop a human prostate cell line model of persistent ZIKV replication, lasting in excess of 30 day and without causing cell death. We also show differences in infection kinetics between clinically relevant, epidemic strains. Additional work is underway to characterize host transcriptional response during ZIKV infection of the male reproductive tract.

Poster Abstracts

252

International Collection of Microorganisms from Plants (ICMP)

Megan Petterson[1], Bevan Weir[1]

1. Landcare Research, St. Johns, AUCKLAND, New Zealand

The International Collection of Microorganisms from Plants (ICMP) is New Zealand's national culture collection of living Bacteria, Fungi, and Chromists. The collection and associated databases considered 'Nationally Significant' by the government, and in part publicly funded.

The ICMP holds 20,000 cultures predominantly from plant, soil, and water in the natural environment, as well as important reference and type cultures of the world's plant pathogenic fungi and bacteria.

All cultures are databased and available online at https://SCD.LandcareResearch.co.nz cultures are available for a fee to cover retrieval costs. New accessions into the collection are welcome, and recommended when publishing papers on microbes to provide a stable permanent resource for future researchers.

The cultures are preserved under liquid nitrogen or in freeze dried ampoules. The ICMP containment and transitional facility conforms to enhanced PC2 Containment criteria, with generic permits to import quarantine and unwanted organisms into New Zealand.

1. Johnston, P. R.; Weir, B. S.; Cooper, J. A. (2017) Open data on fungi and bacterial plant pathogens in New Zealand. Mycology 1-8 doi: 10.1080/21501203.2016.1278409

301

Evaluation of porcine circovirus type 2 (PCV2) infection in pigs by histopathology, IHC and qPCR in tissue and serum samples from the Philippines

Agnes dela Cruz[1], Chiara Palmieri[2], Joanne Meers[2], Conny Turni[3], Tamsin Barnes[2], Christopher Parke[2]

1. The University of Queensland, School of Veterinary Science, Gatton, Queensland, Australia
2. The University of Queensland, School of Veterinary Science, Gatton, Queensland, Australia
3. The University of Queensland, Queensland Alliance for Agriculture and Food Innovation, Ecosciences Precint , Dutton Park, Queensland, Australia

Porcine circovirus type 2 (PCV2) is a significant viral pathogen in pig populations which can cause a multi-systemic syndrome known as porcine circovirus-associated diseases (PCVAD). This syndrome is associated with high economic loss due to decrease in production.

This study aimed to assess PCV2 infection using histopathology, immunohistochemistry (IHC) and quantitative polymerase chain reaction (qPCR) on tissues (mediastinal lymph node, spleen, tonsil and lung) and serum samples collected from slaughtered pigs (N = 47) that were healthy (12/47), had mild or moderate (20/47) and severe (15/47) respiratory signs in the Philippines. Histopathology was performed on haematoxylin-eosin-stained sections, while a specific mouse monoclonal anti-PCV2 antibody (Ingenasa) was used for IHC. qPCR result of $>10^6$ copies/ml of serum was considered consistent with clinical disease.

The following typical characteristic lesions of PCV2 infection were observed in the lymph nodes of eight animals: lymphoid depletion (8/8) and granulomatous inflammation (1/8). Four from these eight pigs were negative for both IHC and qPCR. IHC revealed PCV2 antigen in eight pigs (8/47) in at least one of the tissues: lymph nodes (5), spleen (3), tonsils (4) and lungs (5). PCV2 antigen was observed in three lymph nodes with lymphoid depletion and one lymph node with depletion and granulomatous inflammation. One pig with lymphoid depletion was negative for qPCR but had PCV2 antigen in MLN, tonsil and lung; two pigs with lymphoid depletion had $<10^6$ copies/ml serum - one had PCV2 antigen in MLN and the other had it in spleen and one pig with lymphoid depletion and granulomatous inflammation had $>10^6$ copies/ml serum and had PCV2 antigen in all tissues. One pig showed $>10^6$ as well that did not show histopathology lesions but had PCV2 antigen in spleen and tonsil. Based on these findings, it is suggestive that PCV2 clinical disease is present in the Philippines. Additionally, more samples are currently analysed for further evaluation to see the correlation of the different detection methods used.

302

Peptidoglycan enzymes used by *Salmonella* inside eukaryotic cells

Francisco Garcia-Del Portillo[1], Sónia Castanheira[1], Juan José Cestero[1], Maria Graciela Pucciarelli[3, 2]

1. Centro Nacional de Biotecnologia (CNB)-CSIC, Madrid, Spain
2. Centro de Biología Molecular Severo Ochoa (CBMSO)-CSIC, Madrid, Spain
3. Departamento de Biología Molecular, Universidad Autónoma de Madrid, Madrid, Spain

Salmonella enterica is an intracellular bacterial pathogen that survives and proliferates inside phagocytic and non-phagocytic eukaryotic cells as part of its natural infection cycle. We are interested in deciphering the mechanisms that this pathogen exploits to establish long-lasting persistent infections within eukaryotic vacuolar compartments. We recently discovered that this pathogen controls bacterial progeny by triggering a unique autophagy process involving aggregation of host cell endo-membranes. Our current aim is to dissect envelope alterations that take place in intracellular *Salmonella* and, more specifically, changes in the peptidoglycan (PG) structure that may facilitate a prolonged persistent infection. We have also collected data supporting changes in the PG enzymatic machinery used by *Salmonella* once it colonizes the phagosomal compartment. Some of the PG enzymes up-regulated by *Salmonella* in the phagosome have evolved to act exclusively in this acidic environment and are encoded by genes absent in genomes of non-pathogenic bacteria. These observations support the idea of a dedicated subset of PG enzymes that might promote *Salmonella*adaptation to the intraphagosomal lifestyle. This group of 'specialized' PG enzymes therefore represent new attractive targets to combat intracellular bacterial infections.

303

Changes in susceptibility of oral *Candida dubliniensis* isolates to lysozyme and lactoferrin following brief exposure to drugs with antifungal properties

Arjuna N Ellepola[1], Zia U Khan[2]

1. Oral Microbiology, Faculty of Dentistry, Health Sciences Center, Kuwait University, Kuwait

2. Microbiology, Faculty of Medicine, Health Sciences Center, Kuwait University, Jabriya, Kuwait

Objective: Salivary constituents such as lysozyme and lactoferrin have anti-candidal activity on oral *Candida*. *Candida dubliniensis* is associated with recurrent oral candidiasis. Such *Candida*-associated infections could be managed with drugs with anti-candidal properties such as nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine. *Candida* undergo to a brief exposure to therapeutic agents in the mouth, due to the diluent effect of saliva and the cleansing effect of the oral musculature. There is no evidence on the influence of limited exposure to these drugs with anti-candidal properties on the sensitivity of *C. dubliniensis isolates to* lactoferrin and lysozymes. Hence, this study observed the changes in the sensitivity of *C. dubliniensis isolates to anti-candidal action of* lactoferrin and lysozyme after transitory exposure to sub-lethal concentrations of aforesaid antifungals. ***Materials and Methods****:* After determination of the minimum inhibitory concentration (MIC), twenty *C. dubliniensis* isolates were exposed to sub-lethal concentrations (×2MIC) of nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine for 1 hour. Drugs were removed by dilution and thereafter the susceptibility of these isolates to lysozyme and lactoferrin was determined by a colony forming unit quantification *in-vitro* assay. *C. dubliniensis* CD36 and *C. albicans* ATCC 90028 were used as reference strains. **Results:** Exposure of *C. dubliniensis* isolates to nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine resulted in an increase of susceptibility to lysozyme by 9.45%, 30.82%, 30.04%, 50.64%, 55.60% and 50.18%, respectively ($p < 0.05$ to $p< 0.001$). Exposure of *C. dubliniensis* isolates to nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine resulted in an increase of susceptibility to lactoferrin by 13.54%, 16.43%, 17.58%, 19.60%, 21.32% and 18.73%, respectively ($p < 0.05$ to $p<0.001$). **Conclusion:** Brief exposure to nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine, a scenario all too familiar in the niches of the oral cavity, could enhance the antifungal effect of lysozyme and lactoferrin on *C. dubliniensis* isolates *in vitro*, thereby possibly implicating a synergistic effect, when interacting together. **Acknowledgments:** The work was supported by Kuwait University Research Grant No. DB 01/16.

304

One hour detection of organisms from positive blood culture broth using the BioFire FilmArray® Blood Culture Identification Panel

Catherine Janto[1], Sandra Jones[1], Rifky Balgahom[1], Harsha Samarasekara[1], James Branley[1, 2]

1. Department of Microbiology - Nepean Hospital, NSW Health Pathology, Sydney, NSW, Australia

2. Infectious Diseases and Microbiology, University of Sydney, Sydney, NSW, Australia

Objective

Rapid identification of positive blood cultures enables early directed antimicrobial therapy with associated reduction in mortality and duration of hospital stay. This study assesses the utility of the BioFire FilmArray® Blood Culture Identification (BCID) Panel (bioMérieux) as a qualitative multiplexed PCR assay capable of the simultaneous detection of 24 potential pathogens as well as 3 genes encoding for antimicrobial resistance (*mecA, vanA/B, bla*$_{KPC}$), directly positive blood culture broth.

Methods

Positive blood culture broth with organisms seen by Gram stain (n=70), collected from patients between September 2017 to January 2018 were included in the study and tested according to manufacturer's instructions. All results were compared to routine standard bacterial culture on solid agar plates, bacterial identification of isolated colonies by matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS) using the Bruker MALDI Biotyper® (Bruker Daltonics), and existing in-house PCR for antimicrobial resistance.

Results

Compared to standard culture and in-house PCR methods, 94.2% (66/70) of positive blood culture broths were concordant. 107 targets were detected whereby 96.3% (103/107) were concordant including 10 polymicrobial blood culture specimens. 2 false negatives (*mecA* =1; *E. cloacae* complex =1) and 2 false positives (*E. coli* = 1; *K. pneumoniae* = 1) were observed.

Conclusions

The FilmArray® BCID Panel is able to provide rapid results in approximately 1 hour from when a positive blood culture has flagged positive, providing results approximately 14 hours earlier compared to our existing methods. Based on a review of positive blood cultures at Nepean Hospital in 2017, 86.6% of isolates identified had the potential to be detected on the BCID Panel. Overall we believe it to be a useful tool in aiding early administration of targeted therapy, with the potential to reduce hospital costs and patient stay when used in conjunction with other laboratory and clinical findings.

305

One hour detection of clinically significant pathogens from cerebrospinal fluid using the BioFire FilmArray® Meningitis/Encephalitis Panel

Catherine Janto[1], Rifky Balgahom[1], Harsha Samarasekara[1], James Branley[1, 2]

1. Department of Microbiology - Nepean Hospital, NSW Health Pathology, Sydney, NSW, Australia

2. Infectious Diseases and Microbiology, University of Sydney, Sydney, NSW, Australia

Objective

Meningitis and encephalitis are life threatening conditions which can have serious consequences if not managed early. This study evaluated the effectiveness of the BioFire FilmArray® Meningitis/Encephalitis (ME) Panel (BioMérieux) to provide simultaneous rapid nucleic acid detection of 14 pathogens in cerebrospinal fluid (CSF), including herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human herpes virus 6 (HHV-6), varicella-zoster virus (VZV), enterovirus (EV), cytomegalovirus (CMV), human parechovirus (HPeV), *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Escherichia coli K1, Haemophilus influenzae, Listeria monocytogenes, Streptococcus agalactiae* (GBS), and *Cryptococcus neoformans/gattii*.

Methods

A total of 126 CSF samples were included in this study and tested on the FilmArray® ME Panel according to the manufacturer's instructions. Specimens also underwent routine bacterial culture, and multiplex polymerase chain reaction (PCR) testing using the AusDiagnostics CSF assay and results compared.

Results

The FilmArray® ME Panel detected at least one positive target in 52% (66/126) of specimens tested. Of the targets comparable to the AusDiagnostics CSF assay (HSV-1, HSV-2, VZV, EV, HPeV, *N. meningitidis, S. pneumoniae, H. influenzae, L. monocytogenes,* and *C. neoformans/gattii*), the ME Panel had 98% (124/126) correlation with the reference tests. All targets demonstrated 100% sensitivity and 100% specificity, with the exception of HSV-2 (2 false positive) and *C. neoformans/gattii* (1 false negative). HHV-6 was detected in 4 specimens and confirmed positive by a reference laboratory.

Conclusion

The BioFire FilmArray® ME Panel is highly sensitive and specific assay that provides results in approximately 1 hour. These results were available approximately 24 hours earlier compared to the routine AusDiagnostics CSF assay. Further evaluation is required for CMV, HHV-6, GBS, and *E. coli K1* to assess clinical sensitivity and specificity. The ability to obtain a rapid result may have the potential to allow for early administration of targeted therapy, reducing hospital stay, associated costs and most importantly has the potential to save lives.

306

The prebiotic effects and human gut alteration of Gwakhyangjeonggisan, a traditional Korean medicine

YEONGYUN JUNG[1], Jai-Hyun So[2], Yu-Mi Lim[2], Jae-Ho Shin[1]

1. Kyungpook National University, Daegu, DAEGU, South Korea

2. National Development Institute of Korean Medicine, Gyeongsan, Gyeongsangbuk, Republic of Korea

Gwakhyangjeonggisan (GS) is composed of 11 kinds of medicinal herbs that may affect the human gut microbiome. GS has been used in traditional Korean medicine for the treatment of irritable bowel syndrome with diarrhea. 20 healthy adult volunteers which is composed of 17 males and 3 females were recruited for the experiment. Volunteers took the GS daily for 2 weeks and collected stool samples before and after. Bacterial 16S rRNA gene was amplified from stool metagenomics DNA and sequenced using a next generation sequencing (NGS) machine to analyze the gut microbiota. NGS data were analyzed by bioinformatics programs such as QIIME, PICRUSt, Cytoscape, and Calypso. 2 subjects (10%) complained of diarrhea after taking the traditional medicine, although the unique phylogenetic position of microbiota that previously existed did not change significantly after taking GS. Gut microbiota compositions of these subjects originally showed less than 55% of genera *Prevotella* and *Bacteroides*, which are known to be involved in digestion of food in human gut. "Glycan biosynthesis/metabolism" and "Replication/repair" functions in the gut microbiota of the diarrhea subjects tended to decrease compared to asymptomatic subjects. On the other hand, "Cell motility" and "Membrane transport" tended to increase. In conclusion, GS can negatively affect the human gut microbiota in subjects which have less than 55% of genera *Prevotella* and *Bacteroides*. It is necessary for accumulating database to understand whether the traditional Korean herbs should be prescribed separately according to the human gut microbiota.

1. Mai, Volker, et al. "Monitoring of stool microbiota in subjects with diarrhea indicates distortions in composition." Journal of clinical microbiology 44.12 (2006): 4550-4552.
2. Yang, Yuzhan, et al. "Characterising the interspecific variations and convergence of gut microbiota in Anseriformes herbivores at wintering areas." Scientific reports 6 (2016).
3. Ko, Seok-Jae, et al. "Effect of Korean herbal medicine combined with a probiotic mixture on diarrhea-dominant irritable bowel syndrome: a double-blind, randomized, placebo-controlled trial." Evidence-Based Complementary and Alternative Medicine 2013 (2013).

307

Isolation and initial characterisation of bacteriophages against clinical isolates of *Aeromonas hydrophila*.

Mwila Kabwe[2, 1], Teagan Brown[1], Hiu Tat Chan[3], Steve Petrovski[4], Joseph Tucci[1]

1. Department of Pharmacy and Applied Science, La Trobe Institute for Molecular Science, La Trobe University, Bendigo, VIC, Australia

2. Loddon Mallee Integrated Cancer Service, Bendigo Health Care Group, Bendigo, VIC, Australia

3. Department of Microbiology, Royal Melbourne Hospital, Parkville, VIC, Australia

4. Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, VIC, Australia

Background:

Aeromonas hydrophila causes necrotising disease in mammals and fish. It carries antimicrobial resistant markers and facilitates their movement between fish, poultry and humans. In humans, it causes necrotising fasciitis and sepsis in immunocompromised individuals. Its control is therefore cardinal in health and food security.

Methods:

Wastewater samples collected weekly across Bendigo were screened. Isolated bacteriophages were tested against different clinical *Aeromonas hydrophilia* isolates implicated in sepsis. Initial genetic characterisation of isolated bacteriophages was performed using restriction enzyme digestion on extracted bacteriophage DNA and illumina whole genome sequencing.

Results:

Using a select range of four, five and six base cutters, restriction enzyme digestion revealed three distinct bacteriophages. Full genomic sequences and functional gene characterisation data reveal novel sequences with 85% similarity to published *Ahp1 Podovidiridae* phage. Functional host range analysis has revealed differing lytic capacities and multiplicities of infection against a range of *Aeromonas hydrophilia* isolates. The bacteriophages have shown stability in broth at 4^{o}C for three months with minimal loss of efficacy.

Discussion:

To date there have only been a few reports of lytic bacteriophages against *Aeromonas hydrophilia,* and to our knowledge, those described here are the first isolated, characterised and lytic for clinical isolates of this bacteria. We plan to use *in vivo* mouse sepsis models to assess the capacity for these bacteriophages to lyse *Aeromonas* in the bloodstream. Lytic bacteriophages against *Aeromonas hydrophilia* can be isolated from wastewater and potential exists for testing in the control of *Aeromonas* in agricultural livestock, as well as testing in the treatment of sepsis in humans.

308

Variability of top 7 Shiga toxin-producing *Escherichia coli* (STEC) and microbial populations through slaughter in Australian beef export abattoirs

Seong San Kang, Gary Dykes, Robert Barlow

Australian beef processors must ensure that products exported to the US market are deemed free of regulatory important serotypes of Shiga toxin-producing *Escherichia coli* (STEC). Better understanding of the microbiota and its movement in the abattoir could assist in determining the sites of cross-contamination of top 7 STEC serotypes (O26, O45, O103, O111, O121, O145 and O157) through slaughter. This study aimed to investigate changes in microbial populations and the top 7 STEC during slaughter in two Australian beef export abattoirs with different supply chains. Abattoir A (integrated) and B (fragmented) were visited twice and once, respectively. At each visit 90 samples consisting of 10 faecal samples from the holding pens, 15 from hides, 15 from post-hide pull carcases, 15 from post-evisceration carcases, 15 from pre-chill carcases and 10 environmental samples were collected. Samples were assessed for total viable count (TVC), *E. coli*/coliforms, and traditional (*stx*, *eae* and O-antigens) and novel (*espK* and *espV*) STEC markers. Culture confirmation was conducted on potential positive (PP) samples (*stx*$^{+}$, *eae*$^{+}$ and O-antigen^{+}). Resulting isolates were characterised for traditional and novel STEC markers. TVC of hide samples and pre-chill carcases from abattoir A were significantly higher ($P<0.05$) than abattoir B with an average difference of 0.88 and 0.39 $\log_{10}/cm^2$, respectively. There were no differences between TVC counts for other samples. *E. coli* were present in all hide and faecal samples but were significantly higher ($P<0.05$) in samples from abattoir A than abattoir B. *E. coli* was intermittently present and significantly higher ($P<0.05$) in carcases samples from abattoir A than abattoir B. PP's were identified in 62, 58 (abattoir A) and 58 (abattoir B) samples. PP's decreased to 48, 43 and 36 samples, respectively, with the addition of the novel markers. STEC isolates were only recovered from hide and faecal samples with three O157 and two O111 recovered from all samples tested. Further metagenomics studies will assist in understanding how microbial populations vary through slaughter.

309

Microbial diversity profiling and taxonomy evaluation of macropods using three hypervariable regions of bacterial 16S rRNA gene.

Mohammad Katouli[1], Christian O'Dea[1], Nicole Masters[1], Anna Kuballa[1], Alyce Taylor-Brown[1], Warish Ahmed[2], Helen Stratton[3]

1. University Of The Sunshine Coast, Sippy Downs, QLD, Australia

2. CSIRO, Brisbane, Queensland, Australia

3. Griffith University, Nathan, QUEENSLAND, Australia

We investigated gut microbial diversity profiles and their intra-species variations within several populations of Australian native, eastern grey Kangaroo. Three hypervariable regions of the 16S rRNA gene (V1-V3, V3-V4, and V5-V6) were analysed from faecal samples collected from 30 Eastern Grey Kangaroos (*Macropus giganteus*) across six locations of South-East Queensland. Two methods were used for obtaining the faecal microbial genomic DNA. In the first method, DNA extraction was carried out on composite samples of equal amounts of faecal material of five kangaroos, whereas in the second method the microbial genomic DNA was extracted from each individual kangaroo first and equal amounts of DNA from each kangaroo were pooled into 6 composite samples (five kangaroos each). Both methods used the QiaAmp Powerfaecal DNA extraction kit (Qiagen) for faecal microbial DNA extraction. We obtained 150bp paired-end reads using the Illumina Miseq platform and constructed microbial taxonomic profiles for each variable region. Composite samples containing genomic DNA extracted from individual faecal samples yielded greater intraspecies variability than combining faeces material prior to DNA extraction.

Diversity profiling of gut bacteria using V1-V3 hypervariable region yielded a broader range of taxa due to its longer target region. Higher levels of unassigned taxa were obtained with the V1-V3 regions, these unassigned taxa decreased ≥ 5-fold when analysed using the V3-V4 and V5-V6 regions, which enabled classification of the unassigned into identifiable bacterial taxa. There was insufficient evidence to suggest which hypervariable regions contain the greatest intra-species diversity as each target hypervariable region demonstrated sequence diversity among different bacteria.

310

Comparative genome analyses of *Nocardia seriolae* strains isolated from outbreaks of nocardiosis in fish farms in Vietnam

Mohammad Katouli[1], Cuong Le[1], Derek Sarovich[1], Daniel Powell[1], Erin Price[1], Hung Vu-Khac[2], Dilber Kurtboke[1], Wayne Knibb[1]

1. University Of The Sunshine Coast, Sippy Downs, QLD, Australia

2. 3Central Vietnam Veterinary Institute, , Nha Trang, , Vietnam

Since it was first imported into Vietnam in the 2000s, the pompano fish (*Trachinotus blochii*) has gained popularity as a good candidate for mariculture systems. Between 2010 and 2014, however, several outbreaks of nocardiosis attributed to *Nocardia seriolae* caused massive fish mortalities in pompano farms throughout the country. Based on phenotypic and biochemical properties, 55 strains of *N. seriolae* were isolated from infected pompano in four central Vietnamese provinces. To further understand the origin, evolution and epidemiology of the pathogen, six strains from all provinces were randomly selected and tested for their genetic relatedness using pulsed-field gel electrophoresis (PFGE) and Illumina NextSeq 500 whole genome sequencing (WGS). PFGE with *Xba*I and *Ase*I digestions revealed two pulsotypes with a similarity of > 80%, suggesting close genetic relatedness of these strains. Consistent with PFGE, phylogenomic analysis of the above seven Vietnamese strains and all currently available *N. seriolae* genomes (n=7) using whole-genome-derived single-nucleotide polymorphisms (SNPs) indicated that all seven strains fall into a single clade containing two highly clonal genotypes that differed by just 102 SNPs, irrespective of the geographic regions where they were isolated. The Vietnamese strains share a common ancestor with strains isolated from other countries in the same region, although they differed from the next closest known strain by at least 265 SNPs. These results suggest that the Vietnamese *N. seriolae* strains have been recently introduced to this country, although the precise origin is not yet known. The bacterium encodes a large genome of 7,785,433 bp, a G + C content of 68.2%, 7,420 predicted coding DNA sequences and 77 transfer RNA sequences. It was also found that the bacterium harboured genes coding for virulence factors such as iron uptake systems, resistance to antibiotics and toxic compounds, the biosynthesis of lipid and protein, hemolysins, adhesins and proteases. These findings help focus our future targets for vaccine development against Nocardia in the aquaculture sector and more generally provide novel information about the Vietnamese *N. seriolae* population.

311

Acinetobacter baumannii K24 capsular polysaccharide requires a novel gene for sugar biosynthesis and a gene located outside of the K locus

Johanna J Kenyon[1], Ruth M Hall[2]

1. Queensland University of Technology, Brisbane, QLD, Australia

2. University Of Sydney, CAMPERDOWN, NSW, Australia

Capsular polysaccharide (CPS) is one of the primary virulence determinants for the important nosocomial pathogen, *Acinetobacter baumannii*; acting as an external barrier that protects the organism from antimicrobial compounds, the host immune response, and other external threats. This bacterial surface structure displays significant heterogeneity between different isolates in the species, and 125 different sets of genes have been found at the chromosomal K locus (KL) in an analysis of more than 1000 genome sequences. Our investigation into this genomic region in the multiply antibiotic resistant strain, RCH51, revealed a new gene cluster named KL24. The KL24 gene cluster resembled other *A. baumannii* gene clusters in that it contained genes for nucleotide-sugar biosynthesis, glycosyltransfer, capsule export, and capsule unit translocation (*wzx*), though a *wzy* gene for CPS unit polymerisation was not found. The K24 CPS structure was elucidated by NMR and found to contain D-galactose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, and the rare sugar, 3-acetamido-3,6-dideoxy-D-galactose (D-Fuc3NAc). Three out of five genes for D-Fuc3NAc synthesis were found in KL24. However, an additional gene was found and predicted to encode a new bifunctional protein, FdtE, with a N-terminal 3,4-ketoisomerase domain and a C-terminal acetyltransferase domain, which would replace the activity of the other two proteins in the synthesis pathway. Investigation of the whole genome sequence of RCH51 further revealed a *wzy* gene in a small genomic island (GI) in the same location as a different GI carrying *wzy* found in several other *A. baumannii* isolates. Evidence was found that suggested the GI is likely mobile. Direct correlation of CPS gene cluster content and CPS structural data of these major antigenic structures in *A. baumannii* has proven to be a useful method to predict novel synthesis pathways and confirm the functions of proteins involved in CPS biosynthesis. This knowledge forms the foundations for future biochemical studies.

Poster Abstracts

312

Combating multidrug-resistant bacteria by phages equipped with sRNAs

Karen HK Kong[1], Terrence CK Lau[1]

1. City University of Hong Kong, Hong Kong

Antibiotic-resistant bacterial infection has become an endemic health threat in recent decades. These infections are difficult to be cured by conventional antibiotics, leading to prolonged hospital stays and increased mortality. Antibiotic resistance genes are commonly encoded on either the chromosome or plasmids. Bacterial plasmids are extra-chromosomal circular DNA that are able to replicate independently within bacterial hosts. Transfer of plasmids between bacterial cells via conjugation is an effective approach to disseminate antimicrobial resistance. Inhibiting plasmid replication will be an attractive idea to modify antibiotic-resistant bacteria to drug sensitive.

In this project, we aim to suppress the replication of a multidrug resistance plasmid, pNDM-HK, utilizing the counter-transcribed RNA (ctRNA). pNDM-HK is the first plasmid that encodes New Delhi metallo-β-lactamase (NDM-1), a carbapenemase, in Hong Kong. Previously we has sequenced the pNDM-HK encoded small regulatory RNAs (sRNAs) and proposed NDM-sR2 as the ctRNA to regulate plasmid replication. Herein, we confirmed NDM-sR2 could suppress the expression of replication protein in pNDM-HK. This ctRNA was then integrated into a phage to infect bacteria harboring pNDM-HK. Interestingly, resulting transductants got rid of the drug resistance plasmid after infection. Meanwhile, they became susceptible to various antibiotics including extended-spectrum β-lactamases (ESBLs) and carbapenems. In conclusion, this is the first study to remove a clinical multidrug resistance plasmid with the combination of phage and ctRNA. We believe this approach could be further developed as a therapeutic tool to combat antibiotic-resistant bacteria.

313

A chemoreceptor in *Campylobacter jejuni* responds to multiple classes of chemoeffectors

Bassam A Elgamoudi[1], Ekaterina P Andrianova[2], Christopher J Day[1], Rebecca M King[1], Hossinur Rahman[1], Lauren E Hartley-Tassell[1], Igor B Zhulin[2], Victoria Korolik[1]

1. Institute for Glycomics, Southport, QLD, Australia

2. Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA

Tlp10 is a chemoreceptor in *C. jejuni*, which has a classical transmembrane topology, but its periplasmic ligand-binding domain (LBD) does not match any known domain model and its ligands were unknown. Here, we show that Tlp10 LBD has a bimodular architecture and its predicted secondary structure is reminiscent of the HBM (helical bimodular) domain found primarily in *Pseudomonas*; however, we found no evidence for its homology to HBM or any other helical domain (e.g. four-helix bundle). Using glycan arrays and surface plasmon resonance, we found that Tlp10 LBD binds fucose, galactose, mannose, lactose and Neu5Acα2 (sialic acid), all of which are known to play a role in initial colonisation of the host by the *C. jejuni*. The disassociation equilibrium constant (K_D) of Tlp10 LBD for these glycans ranged between 2.9 μM and 39 μM. Modified nutrient depleted chemotaxis assay was used to reveal that Tlp10 was also able to respond to isoleucine, purine, malic acid, α-ketoglutarate, aspartate and fumaric acid, as attractants, and to arginine and thiamine, as repellents. We identified several highly conserved amino acid residues in Tlp10 LBD (e.g. Y70, D71, F111, K112, N115, N120, S121, and H193) that suggest more than one chemoeffector binding site, which is consistent with the observed multiple chemoreceptor specificity of the Tlp10 chemoreceptor. The evolutionary genomics analysis indicates that Tlp10 LBD was born as a sensory domain in *Pseudomonas* histidine kinases and then propagated to other signal transduction proteins, such as c-di-GMP turnover enzymes and chemoreceptors, and to other phylogenetic groups, such as epsilon-proteobacteria, including *Campylobacter* and *Helicobacter*, as well as cyanobacteria via domain shuffling events and horizontal gene transfer. Tlp10 LBD might be the first example of a sensory domain with specificity to glycans, amino acids, organic acids, and nucleotides.

314

Bacteriophages as a biocontrol agent of *Vibrio* species contaminating algae cultures in aquaculture

Tuan Son Le[1], Paul C. Southgate [2], Wayne O'Connor[3], Daniel Shelley[1], Tomer Abramov[1], Dilber Kurtboke

1. University of the Sunshine Coast, Sippy Downs, QLD, Australia

2. Australian Centre for Pacific Islands Research , University of the Sunshine Coast, Maroochydore, QLD, Australia

3. NSW Fisheries, Port Stephens Fisheries Institute, Taylors Beach, NSW 2316, Australia

One of the potential sources of *Vibriosis* of oyster larvae in hatcheries is contaminated microalgae feed. Antibiotics have been used to prevent the proliferation of Vibrio spp. in hatcheries but this is discouraged due to detrimental effects on the environment and the potential for antibiotic resistant bacteria to develop. Accordingly, the use of bacteriophages as an environmentally friendly biological treatment to control bacterial contamination has value for the shellfish industry. In this study, we demonstrate that bacteriophages can be effectively used in controlling the *Vibrio* spp. contamination on microalgae broth. A *Vibrio alginolyticus* strain, which is pathogenic to oyster larvae, was isolated from an oyster hatchery in Port Stephens Fisheries Institute, NSW, Australia. Four different polyvalent *Vibrio* phages (belonging to *Myoviridae* viral family) were successfully isolated from marine water at the Sunshine Coast region, Australia. The bacteriophages were then applied as a cocktail for the treatment of pathogenic *Vibrio* spp. contaminating a microalgae suspension. Significant decreases in the *Vibrio* spp. concentration were observed within 2 h, suggesting that the direct application of the phages to the aquaculture water could be an effective method in reducing the potential for *Vibriosis* in oyster hatcheries.

Poster Abstracts

315

Whole genome analysis of *Aspergillus sojae* SMF 134 supports its merits as a starter for soybean fermentation

Inhyung Lee[1], Kang Uk Kim[1], Kyung Min Kim[1], Yong-Ho Choi[2], Byung Serk Hurh[2]

1. Bio & Fermentation Convergence Technology, BK21 PLUS project, Kookmin University, Seoul, SEOUL, South Korea

2. Sempio Fermentation Researh Center, Sempio Foods Company, Osongeup, Korea

Aspergillus sojae is a *koji* (starter) mold that has been applied for food fermentation in Asia. Recently, we have isolated the *A. sojae* SMF 134 strain form *meju* (Korean soybean fermented brick) which showed high protease and leucine aminopeptidase (LAP) activities. We analyzed the whole genome of *A. sojae* SMF 134 to evaluate its potential as a starter for soybean fermentation at the genomic level. The strain SMF 134 had total 151 protease genes (including *lap* genes), which were more than *A. oryzae*. According to RNA seq analysis, some endopeptidase genes were highly expressed during soybean fermentation, suggesting that SMF 134 degrades soybean proteins to small peptides efficiently. In addition, the two more *lap* genes were found in addition to the previously known *lap1* and these three *lap* genes were confirmed to be expressed. Three γ-glutamyltranspeptidase (GGT) genes were also identified and a higher GGT activity was observed in *A. sojae* SMF 134 than in *A. oryzae* RIB 40. Because LAP and GGT are known to be important for the flavor development in soybean fermentation, these genome characteristics support that *A. sojae* has great merits as a starter for soybean fermentation.

316

Human brucellosis by *Brucella melitensis* in South Korea

Kyeong Ah Lee[1], Mi Yeon Kim[1], Byung Hak Kang[1], Jae Ok Kim[1], Seon Do Hwang[1]

1. Korea Center for Disease Control and Prevention, Cheongju-si, CHUNGCHEONGBUK-DO, South Korea

Brucellosis is a common zoonotic disease that spreads from animals to human. Human brucellosis is caused by bacteria of the genus *Brucella*. *Brucella abortus, B. melitensis, B. canis, B. suis, B. ovis* remain the principal causes of human brucellosis worldwide and are major public health problem. In Korea, since the first case of human brucellosis in 2002, almost cases have been caused by *B. abortus* and reported over the past 12 years. There was the first official case of human brucellosis caused by *B. melitensis* in Korea in 2015. *B. melitensis* is the most serious brucellosis all over the world. There were 2 cases in 2015, 3 cases in 2016 and 2 cases in 2017. We introduce human brucellosis cases caused by *B. melitensis* in 2015-2017 which were referred to Korea CDC.

Suspected blood samples of brucellosis were sent to Korea CDC and inoculated into media of BACTEC system to cultivate. As a result, 7 of 143 samples were identified as *B. melitensis* in BL3 lab. They were grown in Brucella agar plate and showed negative result for H_2S production and positive reaction for oxidase and urease. Extracted DNA from isolates were amplified in *Brucella* specific genes by multiplex PCR and confirmed this DNA as *B. melitensis* species. In the serologically test on the basis of MAT (microagglutination test), 7 patients presented MAT titers of ≥1:80.

These seven cases identified as *B. melitensis* had clinical history that they had been abroad or got unpasteurized milk or camel milk or contact with sheep. In Korea there has no report of *B. melitensis* in animals until now. Although there was no naturally born *B. melitensis* case in Korea, we need to diagnose any kind of *Brucella* species. The visitors to endemic or epidemic areas with *B. melitensis*need to pay attention not to get contact with cattle, goat and sheep nor eat unpasteurized milk and dairy product.

Acknowledgements: This work was supported by Korea Centers for Disease Control and Prevention
(2016-NG52002-00, 4837-301-210-13, 4838-303-210-13)

317

Mechanisms of intracellular survival of *Pseudomonas aeruginosa* in *Acantamoeba castellanii* and raw 264.7 macrophage cells

Wai Leong[1], Carla Lutz[1], Jia Chi Tan[1], Diane Mcdougald[1]

1. Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

Pseudomonas aeruginosa is an opportunistic pathogen associated with cystic fibrosis lung infections and nosocomial infections. Although it is known to induce the formation of membrane blebs in epithelial cells, it is not a recognised intracellular pathogen, and little is known about its survival within host cells. Previously, we have shown that *P. aeruginosa* is able to survive within *Acanthamoeba castellanii,* and amoeba co-adapted P. aeruginosa is more fit when grown with amoeba compared to the parental strain. Here, we further investigate mechanisms of intracellular survival and replication of *P. aeruginosa* in amoeba and macrophage using confocal microscopy.

GFP-tagged strains were used to visualise cells intracellularly. Data show that the co-adapted P. aeruginosa replicate within amoeba. During infection, infected host cells secrete bacteriocidal compounds such as NO, peroxides, and anti-microbial peptides into phagosomes. Phagosome-lysosome fusion occurs and intracellular bacteria are further challenged by a decrease in pH. In our study, infected host cells were stained with Lysotracker (Thermo, USA) and DAF-FM diacetate (Thermo, USA) to visualise lysosomes and NO respectively. Phagosome acidification and NO secretion were lower within amoeba phagosomes containing the wild type P. aeruginosa compared to *E. coli* OP50. As PA2283 (*mgtC*) was highly upregulated in the transcriptome of intracellular P. aeruginosa, we tested a mutant of PA2283 and demonstrate that intracellular survival was reduced within both amoeba and raw 264.7 macrophage cells. However, phagosome acidification was restored in amoeba but not in macrophage.

318

Unlocking the Complete Arsenal of *Acinetobacter baumannii* Type VI Secretion System Effectors

Jessica M Lewis[1], Timothy C Fitzsimons[1], Deanna Deveson Lucas[1], Marina Harper[1], John D Boyce[1]

1. Department of Microbiology, Infection and Immunity Program, Monash Biomedicine Discovery Institute, Monash University, Clayton, VIC, Australia

Nearly 10% of all nosocomial Gramnegative bacterial infections are caused by *Acinetobacter baumannii,* a strictly aerobic coccobacillus. *A. baumannii* expresses a type VI secretion system (T6SS), a unique surface structure resembling an inverted T4 bacteriophage that is used by *A. baumannii* to inject effectors into nearby cells. In some bacteria, the T6SS is used to inject effectors involved in virulence or biofilm formation, but in *A. baumannii* the T6SS is primarily associated with killing competitor bacteria. In *A. baumannii* strains ATCC17978 and AB3070294, the secretion of each toxic effector is dependent upon a specific VgrG protein. The genes encoding the effector and its cognate VgrG protein are often colocalised on the chromosome. In order to identify novel *A. baumannii*effectors, we analysed the genomes of 41 strains representing a range of *A. baumannii* global clone lineages and sequence types. Several putative effector genes were identified in each genome, each of which were adjacent to a gene encoding a protein with a VgrG-domain. In total, 29 putative *A. baumannii* T6SS effector genes were identified and grouped based on shared domains and the level of amino acid identity. The effectors identified included six Rhsdomain family proteins, each with a unique Cterminal domain of no known function, eight predicted peptidoglycan hydrolases, and three putative nucleases. Moreover, several putative effectors contained a predicted lipase motif. Eight of the putative effectors were novel and had no identifiable domains. Our aim is to characterise the novel effectors via heterologous expression in *E. coli* and *in vitro* functional assays. Characterisation of these novel effectors will provide an insight into bacterial killing mechanisms and potentially allow for the identification of new drug targets.

319

The performance of an oral microbiome biomarker panel in predicting oral cavity and oropharyngeal cancers

Yenkai YKL Lim[1, 2], Naoki NF Fukuma[3], Makrina MT Totsika[1], Mark MM Morrison[4, 2], Lizbeth LK Kenny[5], Chamindie CP Punyadeera[1, 2]

1. The Institute of Health and Biomedical Innovation, The Queensland University of Technology, Brisbane, QLD, Australia

2. The Translational Research Institute, Brisbane, QLD, Australia

3. The Department of Life and Food Science, The Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan

4. The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia

5. Royal Brisbane and Women's Hospital, Brisbane, QLD, Australia

Introduction: The oral microbiome plays a role in the instigation of oral diseases that have been linked to various systemic conditions of humans. This study explored oral microbiome fluctuations and their association with oral cavity (OCC) and oropharyngeal cancers (OPC) [1].

Methods: The study cohort consists of normal healthy controls (n = 10, between 20 to 30 years of age; n = 10, above 50 years of age), high-risk individuals (n = 11, above 50 years of age with bad oral hygiene and/or oral diseases) and OCC and OPC patients (n = 31, HPV-positive; n = 21, HPV-negative). Oral rinse samples were analysed using 16S rRNA gene amplicon sequencing on the MiSeq platform. Kruskal-Wallis rank test was used to identify genera associated with OCC and OPC. A logistic regression analysis was carried out to determine the performance of these genera as a biomarker panel to predict OCC and OPC [2]. In addition, a two-fold cross-validation with a bootstrap procedure was carried out in R to investigate how well the panel would perform in an emulated clinical scenario [3].

Results: Our data indicate that the oral microbiome is able to predict the presence of OCC and OPC with sensitivity and specificity of 100% and 90%, respectively.

Conclusion: With further validation, the panel could potentially be implemented into clinical workflow as OCC and OPC diagnostic and prognostic biomonitors.

1. Lim, Y., et al., Oral Microbiome: A New Biomarker Reservoir for Oral and Oropharyngeal Cancers. Theranostics, 2017. 7(17): p. 4313-4321.
2. Shultz, E.K., Multivariate receiver-operating characteristic curve analysis: prostate cancer screening as an example. Clin Chem, 1995. 41(8 Pt 2): p. 1248-55.
3. Lim, Y., et al., Salivary DNA methylation panel to diagnose HPV-positive and HPV-negative head and neck cancers. BMC Cancer, 2016. 16(1): p. 1-12.

320

Global Metabolomic Analysis of the Synergistic Killing against Extensive Drug-resistant *Pseudomonas aeruginosa* by the Combination of Polymyxin B and Enrofloxacin

Yu-Wei Lin[1], Mei-Ling Han[1], Jinxin Zhao[1], Darren Creek[2], Yan Zhu[1], Tony Velkov[3], Jian Li[1]

1. Monash University, Clayton, VIC, Australia

2. Monash University, Parkville, Vic, Australia

3. The University of Melbourne, Melbourne, Vic, Australia

Background: Polymyxins are increasingly used as a last-resort therapy against extensive drug-resistant (XDR) *Pseudomonas aeruginosa*[1]. However, polymyxin resistance can rapidly emerge with monotherapy[2]. Synergistic antibiotic combination therapy holds great promise in maximising bacterial killing and minimise the emergence of resistance[3]. The combination of polymyxin B and enrofloxacin has been shown to display synergistic killing against XDR *P. aeruginosa*[4]. We employed a global metabolomic analysis to investigate the synergistic killing mechanism of polymyxin B (PMB) and enrofloxacin combination therapy against XDR *P. aeruginosa*.

Poster Abstracts

Methods: An XDR isolate *P. aeruginosa* 12196 was treated with clinically relevant concentrations of PMB (2 mg/L) and enrofloxacin (1 mg/L) alone or in combination. Metabolome profiles were obtained with bacterial samples collected at 1, 4 and 24 h using LC-MS, and analysed using univariate and multivariate statistics via MetaboAnalyst (v 4.0). Significantly perturbed metabolites ($q<0.05$, $Log_2FC\geq2$) were subjected to pathway analysis using BioCyc.

Results: At 1 h PMB monotherapy induced potential membrane remodelling as indicated by perturbation of glycerophospholipid (e.g., *sn*-glycero-3-phosphoethanolamine) and fatty acid metabolites, whereas that with enrofloxacin monotherapy induced minimal metabolomics perturbations. Compared to PMB or enrofloxacin monotherapy, PMB/enrofloxacin combination therapy had dramatic effects on a greater number of metabolic pathways at 4 and 24 h. These included the pentose phosphate pathway, pyrimidine and purine ribonucleotide biogenesis, glycerophospholid biogenesis, peptidoglycan biogenesis, lipid and energy metabolism. Moreover, the increased glutathione (log_2FC =5.01) and glutathione disulphide (log_2FC = 2.41) levels indicated that PMB/enrofloxacin therapy induced oxidative stress to bacterial cells. Importantly, compared to PMB monotherapy the combination therapy significantly minimised PMB resistance via the inhibition of lipid A modification at 24h.

Conclusions: This is the first study to elucidate the synergistic mechanism of PMB/enrofloxacin against XDR *P. aeruginosa*. Metabolomic analyses provide crucial information to understanding mechanisms of activity, resistance and synergism of antibiotics at a systems level. Future integration of metabolomics data with pharmacokinetic/pharmacodynamic model will help to optimise dosing regimen in humans.

1. Li J, Nation RL, Turnidge JD et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. The Lancet Infectious Diseases 2006; 6: 589-601.
2. Cheah S-E, Li J, Bergen PJ et al. Polymyxin Pharmacokinetics and Pharmacodynamics. In: Rotschafer JC, Andes DR, Rodvold KA, eds. Antibiotic Pharmacodynamics. New York, NY: Springer New York, 2016; 221-60.
3. Bergen PJ, Bulman ZP, Saju S et al. Polymyxin combinations: pharmacokinetics and pharmacodynamics for rationale use. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 2015; 35: 34-42.
4. Lin Y-W, Yu HH, Zhao J et al. Polymyxin B in Combination with Enrofloxacin Exerts Synergistic Killing against Extensive Drug-resistant Pseudomonas aeruginosa. Antimicrobial Agents and Chemotherapy 2018.

321

The carbohydrate dimension of nutrition in gut microbiome modulation

Alison WS Luk[2, 1], Laurence Macia[2, 1], David Raubenheimer[2, 1], Andrew Holmes[2, 1]

1. Centre for Advanced Food Enginomics, The University of Sydney, Camperdown, NSW, Australia

2. Charles Perkins Centre, The University of Sydney, Camperdown, NSW, Australia

Dietary carbohydrates impact multiple host systems, including gut functions, the gut microbiome and immunometabolic health. There is great interest in promoting microbial-dependent health benefits through manipulating the carbohydrate profile of food, especially by increasing dietary fibre content. However, microbial and host responses to dietary fibre supplementation show unpredictable interindividual variation. We hypothesise that different types of dietary fibre and the interactive effects with other diet components shape microbial interactions.

To test this hypothesis, we functionally categorised carbohydrates by host- and microbial-accessibility and designed ten carbohydrate profiles by altering the proportions of glucose and sucrose to resistant starch type II and type III to inulin and guar gum. Using a mouse model, we systematically investigated these carbohydrate profiles at two macronutrient compositions (high prot:carb or high carb:prot), and two absorbable energies (high or low levels of indigestible carbohydrates), in a total of 40 experimental diets. Gastrointestinal transit was inferred through faecal and caecal attributes, and the presence of faecal blood was used as an indicator of gut epithelial health. The gut microbiota was analysed by 16S amplicon profiling using faecal samples.

Intake of host-inaccessible microbial-accessible carbohydrates (HI-MAC) was significantly correlated with increased faecal moisture content ($p<0.01$) and caecal size ($p<0.05$). Diets with high HI-MAC were also associated with reduced faecal blood. Notably, the same carbohydrate profiles had distinct impacts when diets differed in protein content. The microbial composition was driven by the carbohydrate profile, but the overall outcomes were dependent on the dietary context. The relative abundance of *Bifidobacterium* increased with high intake of HI-MAC, but only in diets with low protein intake. The microbial interaction network also differed depending on the diet context, with *Bifidobacterium* as the most interconnected at high carb:prot diets, and *Allobaculum* at high prot:carb.

We conclude that not only is the type and availability of carbohydrates important, but understanding the interactions between multiple dietary dimensions is essential in predicting microbial responses and health effects.

322

Searching for the remarkable among the unremarkable: Neglected plants from the New England region (NSW) and their antibacterial compounds

Dane Lyddiard[1], Ben W Greatrex[1]

1. School of Science & Technology, University of New England, Armidale, NSW, Australia

A variety of antibacterial drug discovery approaches are being undertaken by researchers to fill the treatment gaps left by growing antibiotic resistance. As plants rely heavily on small molecules to protect them from microbial invasion, one approach is to screen the botanical world for antibacterial compounds. While some plant species, such as *Melaleuca alternifolia* (the source of tea tree oil), have garnered significant interest for potential antibacterial uses in the past, many species remain neglected. A selection of plants growing in the New England region of NSW, which had been poorly studied for antibacterial phytochemistry, were screened leading to the discovery of an active methanol extract from an endemic species, *Olearia* aff. *elliptica*. Thin layer chromatography bioautography and activity-guided partitioning and fractionation led to the isolation of known and new compounds (such as *ent*-labdane glycosides and an *ent*-labdane diacid), including those with antibacterial properties. Further work is being undertaken to assess the extent of antibacterial activity and the toxicity of isolated compounds. The limitations of phytochemical screenings are reaffirmed: the problem of 'rediscovering' known compounds, toxicity concerns and the prevalence of phytochemicals with low to moderate antibacterial activity.

323

Characterisation of a Novel Bacteriophage and its Use in Therapies Against Bacteria Associated with Bovine Mastitis.

Stephanie Lynch[1], Karla Helbig[1], Steve Petrovski[1], Travis Beddoe[1], Daniel Rice[1]

1. Department of Physiology, Anatomy and Microbiology , La Trobe University, Melbourne, Victoria, Australia

The Australian dairy industry constitutes a major part of the agricultural sector and contributes a net profit of $35 billion per annum. However, disease within the dairy industry imposes significant economic losses each year. The most prevalent disease within the dairy industry is Bovine Mastitis. Currently, mastitis is treated through antibiotic therapy, which is losing effectiveness due to its misuse, thus resulting in antibiotic resistance. Recent research has shifted to alternative therapeutics to treat bovine mastitis, with bacteriophages receiving the most attention.
This study aimed to further characterise bovine mastitis associated bacteria and aid in the development of more effective phage therapies against this devastating disease. Raw bovine milk and teat skin samples from mastitis positive cows were obtained from farms within the rural Victorian area. The samples demonstrated a high diversity of bacteria, with many being identified as unique genera and not those typically associated with bovine mastitis. Of the bacteria isolated, many possessed high resistance to the three most common antibiotics used to treat bovine mastitis; ampicillin, penicillin and streptomycin. Two bacteriophages were isolated against two separate bovine mastitis-associated bacterial pathogens, both belonging to the Siphoviridae family, with one bacteriophage being novel.
These results highlight the diversity of bacteria associated with bovine mastitis and the high prevalence of antibiotic resistance among these pathogens. This study presents the possibility of phage therapy as an alternative therapy. However, further work is required before this therapy may be applied in a large scale across the dairy industry.

324

Statins promote clearance of Alzheimer's amyloid beta fused to green fluorescent protein in yeast

Ian Macreadie[1], Ken Gardiner[1], Mishal Subhan[2], Rani Faryal[2]

1. RMIT University, Bundoora, VIC, Australia

2. Quaid-i-Azam University, Islamabad, Pakistan

A large-scale epidemiology study on statins previously showed that simvastatin was unique among statins in reducing the incidence of dementia. Since amyloid beta (Aβ) is the protein that is most associated with Alzheimer's disease, this study has focused on how three statins, lovastatin, simvastatin and atorvastatin, influence the turnover of Aβ fused with green fluorescent protein (GFP), in the simplest eukaryotic model organism, *Saccharomyces cerevisiae*. Flow cytometry was employed to evaluate the proportion of cells having GFP fused to Aβ. All statins reduced levels of Aβ fused to GFP, however, simvastatin promoted the greatest clearance. A comparison with fluconazole, which targets that same pathway, suggests that effects of ergosterol synthesis may not account for the clearance of Aβ fused to GFP. This is the first report of statins being involved in clearance of Aβ fused to GFP, providing new insights into how simvastatin exerts its neuroprotective role.

325

Non-capsular antibodies reduce pneumococcal colonisation density following therapeutic administration of pneumococcal whole cell vaccine

Jayne Manning[1, 2], Viktoria R Bongcaron[1, 3], Eileen M Dunne[1, 3], Casey L Pell[1], Polly Burbridge[4], David Goldblatt[4], Kim Mulholland[1, 5], Roy M Robins-Browne[6, 2], Paul Licciardi[1, 3], Richard Malley[7], Odilia L Wijburg[1, 2], Catherine Satzke[1, 3, 2]

1. Pneumococcal Research, Murdoch Children's Research Institute , Parkville, Victoria, Australia

2. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia

3. Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia

4. Institute of Child Health, University College London, London, United Kingdom

5. London School of Hygiene and Tropical Medicine, London, United Kingdom

6. Infectious Diseases and Microbiology, Murdoch Children's Research Institute, Parkville, Victoria, Australia

7. Division of Infectious Diseases, Boston Children's Hospital, Boston, USA

Streptococcus pneumoniae (the pneumococcus) is an important paediatric pathogen that causes a range of respiratory tract diseases and commonly resides in the nasopharynx of young children. Over 90 pneumococcal serotypes exist, each defined by their immunologically distinct capsular polysaccharide antigen. Current pneumococcal conjugate vaccines induce protection against only 10-13 serotypes. The pneumococcal whole cell vaccine (WCV) induces immunity against non-capsular antigens, and protects mice against nasopharyngeal colonisation via antibody-independent, T_H17 mechanisms when administered *before* infectious challenge. In high disease-burden settings infants are exposed to pneumococci soon after birth.Therefore, this project investigated the effect of WCV on colonisation when administered *after* established colonisation ("therapeutic" vaccination). Infant mice were colonised with pneumococcal strain EF3030 (serotype 19F) before subcutaneous immunisation with WCV or adjuvant-only control. Therapeutic WCV significantly reduced the density of pneumococcal colonisation in a dose dependent manner by 4.3-fold (one dose, $p<0.0001$) and 8.6-fold (two doses, $p=0.014$) compared to therapeutic adjuvant (Mann-Whitney). This reduction was dependent on non-capsular antibodies: therapeutic WCV did not affect colonisation in antibody-deficient $\mu MT^{-/-}$ mice compared to therapeutic adjuvant, and levels of serum IgG specific for the WCV antigen ($p=0.003$) and pneumococcal proteins PspA ($p=0.004$), CbpA ($p=0.022$) and PiaA ($p=0.028$) were inversely correlated with colonisation density in wild type mice (Spearman's correlation). No associations between systemic, local or splenic IL-17A responses and colonisation density were observed. In addition, shedding of pneumococci from the upper respiratory tract of mice was reduced after each WCV dose ($p<0.0001$, Mann-Whitney). This project has demonstrated that therapeutic WCV immunisation effectively reduces pneumococcal colonisation and has identified a novel role for non-capsular antibodies in controlling colonisation density. Given that WCV reduces colonisation density and shedding, it is likely that WCV could reduce pneumococcal transmission and contribute to herd protection. These data support further investigation of WCV in high disease-burden settings where children are more likely to carry pneumococci at the time of vaccination.

Poster Abstracts

326

Disrupting and killing of Methicillin-resistant *Staphylococcus aureus* in biofilms

Arthika Manoharan[1], Ashishkumar Das[1], Trevor Glasbey[2], Gregory Whiteley[2], Jim Manos[1]

1. Infectious Diseases and Immunology, The University of Sydney, Sydney, NSW, Australia

2. Whiteley Corporation, Tomago, NSW, Australia

INTRODUCTION: The number of methicillin resistant *S. aureus* (MRSA) cases is increasing globally, with nearly 19000 deaths in the US alone in 2006. Coupled with the spread of vancomycin resistant strains, an alternative therapeutic approach is urgently needed. MRSA is frequently associated with severe biofilm infections, hence targeting the biofilm structure to potentially restore antibacterial susceptibility in MRSA is a promising area of study. We investigated the potential of an innovative combination therapy (CT) in treating MRSA and MSSA. This multi-targeted approach first degrades the extracellular polymeric matrix using an antioxidant and enzyme followed by appropriate antibiotic treatment.

METHODS and MATERIALS: MICs of potential CT formulae were first identified for MRSA and MSSA strains by testing different compositions and dosages on planktonic cultures. The effect of CT on MRSA and MSSA isolates was then determined by measuring biofilm viability using a resazurin assay. This was complemented with confocal microscopy to provide a detailed view of the proportion of live and dead bacteria in treated biofilms, as well as biofilm volume and thickness. Colony counts were conducted to enumerate remaining live bacteria post-treatment. Experiments were then repeated with antioxidants buffered to physiological pH. Fifteen different CT combinations were tested.

RESULTS: Data showed successful disruption of MRSA and MSSA biofilms in vitro. Formulations consisting of glutathione and an antibiotic of choice showed >99% inhibition of bacterial growth in planktonic culture and >95% disruption of preformed biofilm. Furthermore, a 1200-6300 fold decrease in cfu/ml was recorded after treatment. When the antioxidants were buffered to pH 7.4, a 10-50% decrease in biofilm viability occurred, depending on the antioxidant used.

CONCLUSIONS: The CT combinations tested show good promise in disrupting MRSA and MSSA biofilms. Further qualitative and quantitative investigations into the effects of CT on MRSA and MSSA biofilms are underway, including real-time observation of CT activity on biofilms as well as cytotoxicity studies on mammalian cells. Additionally different antioxidants have different targets, hence research is continuing to determine the most effective formulae and dosage.

327

A long-term efficacy trial of a live, attenuated *Salmonella* Typhimurium vaccine in layer hens

Andrea R McWhorter[1], Kapil K Chousalkar[1]

1. University of Adelaide, Roseworthy, SA, Australia

Salmonella remains one of the most common causes of bacterial foodborne gastrointestinal disease in humans. Raw eggs or food items containing undercooked eggs are frequently identified as the source of *Salmonella*. *Salmonella* Typhimurium contamination of table eggs most commonly occurs when they are laid in a contaminated environment. Several control strategies, including vaccination, are widely used to mitigate the total *Salmonella* load. It is unclear, however, whether live attenuated *Salmonella* vaccines are efficacious over the life span of a layer hen. Live attenuated *Salmonella* vaccines have been favoured due to their ability to illicit a strong humoral immune response. The lifespan of a layer hen ranges between 60 and 80 weeks and the long term efficacy of attenuated vaccine strains has not been investigated. In this study, commercial brown layer chicks were vaccinated at day old, 6 weeks of age, and again at 10 weeks of age with the Bioproperties Vaxsafe™ STM1 *aroA* mutant vaccine. Birds were challenged at 18 weeks of age with *Salmonella* Typhimurium DT9 (MLVA 03 15 08 11 550). Feces and eggs were monitored for *S.* Typhimurium for 40 weeks post-infection. Birds produced a strong immune response following the final dose which was administered intramuscularly. The serum antibody response to *S.* Typhimurium DT9 infection did not differ between challenged groups. Fecal shedding and egg contamination was highly variable and did not differ significantly between vaccinated and unvaccinated birds that had been challenged with *S.* Typhimurium DT9. Total bacterial load in feces was quantified using qPCR. No significant difference was detected between unvaccinated and vaccinated birds after challenge.

328

Exploring the behaviour of *Salmonella* Typhimurium in aioli, a raw egg-based sauce

Andrea R. McWhorter[1], Talia S Moyle[1], Kapil K Chousalkar[1]

1. University of Adelaide, Roseworthy, SA, Australia

Salmonella spp. remains one of the most common causes of foodborne gastrointestinal disease. Raw or undercooked eggs or food items containing raw eggs are commonly identified as sources of *Salmonella*. Based on previous research, acidification and cold storage are utilised to prevent bacterial growth and limit survival, yet *Salmonella* is often isolated from mayonnaise and aioli during trace back investigations. In the present study, the effect of temperature and pH on the culturability of *Salmonella* Typhimurium in freshly prepared aioli was investigated. Bacteria were grown overnight either in Luria Bertani or minimal M9 media and diluted to a concentration of 10^5 CFU/ gram aioli prepared at pH 3.5, 4.0, 4.5, or 5.0. Samples were incubated at 5°C and 25°C and bacteria were tested at 0, 4, 8, 12, 24, 48, 72, and 96 hours post-inoculation. At 5°C, the total amount of culturable *Salmonella* in aioli pH 3.5 and 4.0 decreased between 4 and 8 hours post inoculation, with no bacteria cultured subsequently. At higher pH (4.5 and 5.0), *Salmonella* was cultured until 72 hours. At 25°C, *Salmonella* was not cultured at any time point from aioli pH 3.5 or 4.0; at pH 4.5 and 5.0, however, bacteria were cultured at 4 and

8 hours. Increasing the inoculum (10^9 CFU/gram pH 3.5) extended the culturability time at both 5°C and 25°C. Bacteria grown in non-nutritive M9 media exhibited a similar pattern at both temperatures. The bactericidal effects of lemon juice and vinegar were also investigated. Different ratios of lemon juice and vinegar were used to create aioli pH 3.5 but no effect on culturability of *Salmonella* was observed. Live/dead staining on aioli preparations where bacteria were no longer culturable revealed that the majority of the bacteria were alive. Aspects of bacterial virulence, including motility and invasiveness into cultured intestinal epithelial cells are currently being investigated.

329

Molecular characterization of porcine circovirus type 2 in South East Queensland pig herds, Australia

Nway K. Mone[1], Conny Turni[2], Myat Kyaw-Tanner[1], Tamsin S. Barnes[1], Christopher R. Parke[1], John Al. Alawneh[1], Pat J. Blackall[2], Joanne Meers[1]

1. The University of Queensland, Gatton, QLD, Australia

2. Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, Brisbane, QLD, Australia

Porcine circovirus type 2 (PCV2) is of major economic importance in the pig industry globally. PCV2 has a high rate of evolution among DNA viruses and four distinct genotypes have been identified: PCV2a, PCV2b, PCV2c and PCV2d[1,2]. This study aims to determine the occurrence of these genotypes in Australia.

Lymph node samples were collected at slaughter from 200 pigs with pleurisy derived from 44 farms and blood samples were collected from 30 live pigs from another farm. Serum and lymph nodes were stored at -20°C until kit-based DNA extraction. PCV2 status of samples was determined in a qPCR assay3. Of the lymph node and serum samples, 85 (42.5%) and 25 (83.3%) were PCV2-positive, respectively. Eleven PCV2-positive DNA samples with high virus load were subjected to conventional PCR to amplify the whole genome of PCV2 (1768 bp), followed by sequencing. The DNA samples were derived from 9 lymph node samples from 6 farms and 2 serum samples from one farm. The sequences were aligned using the Clustal W and phylogenetic analysis was performed using Neighbouring Joining method in the ClwstalW on MEGA7 program, with reference sequences from NCBI and GenBank databases.

The majority (9 out of 11) of the analysed-PCV2 sequences were PCV2a or PCV2b genotypes and clustered with other Australian sequences. Interestingly, the 2 remaining sequences clustered with PCV2d genotype, which has not yet been reported in Australia. In 4 farms each with 2 samples analysed, sequence variation within farm was very low.

This study is in agreement with previous studies that found PCV2a and PCV2b are the predominant genotypes in Australia, but has shown that PCV2d is also present in this country. PCV2d is currently a fast-spreading genotype globally, with reported high virulence. The extent of the distribution of the PCV2d genotype in Australia is unknown. The potential implications of these findings with respect to pathogenicity and vaccine efficacy require further investigation.

1. Segales J, Kekarainen T, Cortey M. Vet Microbiol 2013;165:13-20.
2. Opriessnig T, Meng X-J, Halbur PG. Journal of Veterinary Diagnostic Investigation 2007;19:591-615.
3. Olvera A, Sibila M, Calsamiglia M, Segalés J, Domingo M. Journal of virological methods 2004;117:75-80.

330

Evolutionary changes in the capsid P2 region of the norovirus GII.Pe_GII.4

Jean M Moselen[1], Leesa D Bruggink[1], Jason A Roberts[1], John A Marshall[1]

1. Victorian Infectious Diseases Reference Laboratory, Melbourne, VICTORIA, Australia

The noroviruses are now considered the most common cause of outbreaks of nonbacterial gastroenteritis. The norovirus virion comprises 90 protein dimers of the norovirus capsid protein, each of which has two domains: the shell domain and the protruding (P) domain. The shell domain is involved in the formation of the icosahedral shell whereas the P domain forms arch-like protrusions. The P domain has been divided into the P1 subdomain and the P2 subdomain, with the P2 region of the capsid thought to be involved in receptor binding and immunogenicity and includes documented hypervariable sites.

This study examines the changes that occurred in the P2 region of GII.Pe_GII.4 norovirus in the course of its evolution from a precursor phase (2008-2009), to an intermediate phase (2010) and finally to an epidemic phase (2012-2015).The findings are reviewed in relation to the progressive evolution of the virus and to previously documented hypervariable sites in the P2 region.

Twenty-two P2 region amino acid (aa) sequences (166 aa long) from all phases of the evolution of the virus were compared and the changes analysed. 3D computer modelling of the capsid proteins was performed to aid in locating the variable sites on a norovirus capsid protein.

Twenty sites in the P2 region underwent change and, of these, ten corresponded to previously proposed hypervariable sites and ten to novel hypervariable sites. It was notable that aa changes at two sites, X and Y, only emerged as the epidemic phase progressed. 3D modelling showed their location to not be on the exposed surface of the virion and combined with the nature of the aa changes suggests these sites were important in enhancing the structural integrity of the capsid, which in turn may have facilitated the longer term viability of the virus.

The current study helps establish the validity of previously proposed hypervariable sites in the P2 region as well as indicating new ones. It also provides data on how these sites changed over the evolutionary history of a particular norovirus strain. Studying how epidemic strains of norovirus evolve may assist in predicting future norovirus epidemics.

1. Bruggink L, Moselen J, Roberts J & Marshall J (2017) Evolutionary changes in the capsid P2 region of Australian strains of the norovirus GII.Pe_GII. 4. J. Med. Microbiol. 66: 1014-1022.

331

Organoids, a new, innovative way to grow “unculturable” human viruses that pose a public health threat

Jean M Moselen[1, 2], Renate HM Schwab[2], Bang M Tran[2], Dustin J Flanagan[2], Christopher Christophi[3], Theodora Fifis[3], Leon Caly[1], Elizabeth Vincan[1, 2]

1. Victorian Infectious Diseases Reference Laboratory, Melbourne, VICTORIA, Australia

2. University of Melbourne, Melbourne, Victoria, Australia

3. Department of Surgery, University of Melbourne, Melbourne, Victoria, Australia

Organ-restricted adult stem cell derived 3D replicas of organs grown in tissue culture, termed organoids, have led to remarkable advances in stem cell and developmental biology, cancer and regenerative medicine. Organoids are now poised to advance our understanding of infectious disease because they accurately recapitulate the essential function and architecture of intact tissues. Our aim is to establish organoids to models natural infection by pathogens of public health importance.

Using protocols established by the Clevers laboratory in the Netherlands, we have successfully established organoids from mouse small intestine, colon and liver; and human colon and liver. Briefly, the stem cells compartments of these tissues are resuspended in an extracellular matrix (Matrigel), and seeded into tissue culture plates. Once the Matrigel has set, it is covered with medium containing growth factors necessary for stem cell maintenance and tissue organization. The stem cells self-organise into organoids giving rise to all the cells type normally derived from the stem cell. Using immunofluorescence and confocal microscopy, quantitative RT-PCR and immunohistochemistry, we have characterised gut and liver organoids and demonstrated that they faithfully recapitulate adult tissue. We have demonstrated that material can be microinjected into the lumen of organoids, and that we can establish organoids from diseased tissues such as human cancer. Furthermore, the organoids are readily genetically manipulated to study gene function.

Tissue-restricted stem cell derived organoids recapitulate the characteristics of the intact adult tissue and are readily infected with viruses that only infect specific adult human cell types. We will now expand our tissue repertoire to include lung organoids as infection of the respiratory tract, gut and liver accounts for the majority of human infectious diseases, globally.

332

The effect of *in vivo* passage on *S.* Typhimurium MLVA type and virulence

Talia S Moyle[1], Andrea R McWhorter[1], Kapil K Chousalkar[1]

1. School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy, SA, Australia

Majority of Australian *Salmonella* Typhimurium (*S.* Typhimurium) outbreaks are linked with the consumption of contaminated eggs or egg products. Multi Locus Variable Number Tandem Repeat Analysis (MLVA) has been the preferred method for trace back investigation of outbreaks. The aims of this study were to determine if *in vivo* passage of *S.* Typhimurium in laying hens led to changes in MLVA pattern and whether there were subsequent changes in virulence. Hens were infected at 14 weeks post hatch with either 10^9 colony forming units (CFU) of *S.* Typhimurium or 10^9 CFU of an equal suspension of both *S.* Typhimurium and *S.* Mbandaka. Faecal samples were collected from each bird at days 1, 3, 6, 9, 12 and weeks 3, 5, 7, 9, 11, 13 and 15 post-infection (p.i.). Faecal shedding of *S.*Typhimurium was highest in both treatment groups at day 6 p.i. and decreased from there on. Shedding was significantly higher in the single infection group ($P \leq 0.01$). MLVA typing of *S.* Typhimurium isolates collected during the trial revealed a change in MLVA pattern at week 9 p.i. onwards in bacteria isolated from the co-infection treatment group (from 03-24-11-11-523 to 03-24-12-11-523). Human intestinal epithelial cells (Caco2) were used for invasion assays, with this assay being designed to characterise potential changes in invasion, one aspect of bacterial virulence. No statistically significant difference in invasion was observed between different MLVA types, however percent invasion was variable across all isolates. The change in MLVA pattern occurred after only one *in-vivo* co-infection passage of the isolate thus, the co-infection environment may be responsible for driving MLVA change.

333

Lectin activity of the Bexsero *Neisseria meningitidis* serogroup B vaccine antigen NHBA

Tsitsi D Mubaiwa[1], Lauren Hartley-Tassell[1], Evgeny A Semchenko[1], Christopher J Day[1], Michael P Jennings[1], Kate L Seib[1]

1. Institute for Glycomics, Griffith University, Gold Coast, QLD, Australia

Neisseria meningitidis is a Gram-negative bacterial pathogen that causes life threatening meningitis and septicemia. Neisseria Heparin Binding Antigen (NHBA) is an outer membrane protein that binds heparin and heparan sulfate and DNA. This protein is one of the four antigens in the meningococcal serogroup B vaccine Bexsero. In the current study, we sought to define the full glycan-binding repertoire of NHBA to better understand its role in meningococcal pathogenesis and vaccine efficacy. Glycan array analysis revealed binding to 28 structures by recombinant NHBA. Surface plasmon resonance was used to confirm the binding phenotype and to determine the affinity of the interactions. These studies revealed that the highest affinity binding of NHBA was with chondroitin sulfate ($K_D = 5.2$ nM). This affinity is 10-fold higher than observed for heparin. Analysis of binding with well-defined disaccharides of the different chondroitin sulfate types demonstrated that the most preferred ligand has a sulfate at the 2 position of the GlcA/IdoA and 6 position of the GalNAc, which is an equivalent structure to chondroitin sulfate D. Chondroitin sulfate is widely expressed in human tissues, while chondroitin sulfate D is predominantly expressed in the brain and may constitute a new receptor structure for meningococci.

334

Meningococcal Carriage Study of Young Adults in Western Australia

Christopher A Mullally[1], Lauren Bloomfield[2, 3], Shakeel Mowlaboccus[1], Paul Effler[3], Charlene M Kahler[1, 4]

1. University of Western Australia, Perth, W.A., Australia

2. Edith Cowan University, Perth, Western Australia, Australia

3. Department of Health, Perth, Western Australia

4. Telethon Kids Institute, Perth, W.A., Australia

Neisseria meningitidis is the causal agent of epidemic bacterial meningitis and sepsis. It is carried asymptomatically in the nasopharynx of healthy individuals but can occasionally cross the mucosal membrane resulting in life threatening illness. In 2017, the rate of invasive meningococcal disease notifications in Australia was 1.6 per 100 000, the highest since 2006. This increase in disease notifications is overwhelmingly due to an increased prevalence of serogroup W strains. The highest risk demographic are infants under the age of one and adolescents aged 15-21 years. This is due to immature immune response in infants, and higher transmission and carriage rates of meningococci in adolescents compared to the rest of the population.

In 2017, Western Australia, commenced a quadrivalent A/C/W/Y vaccination program targeting 15-19 year-old adolescents. Despite this vaccination programme, the rate of meningococcal notifications did not decrease with a total of 20 notifications as of 31st January 2018. A pilot meningococcal carriage study was commenced to determine the carriage rate of at risk individuals as a prelude to a larger state-based vaccination intervention in this age group to commence in August 2018.

Pharyngeal swabs were collected from 400 university students primarily aged 21-25 years. These swabs were used to inoculate GC-Lect selective media, and colonies that grew after 18 hrs incubation were tested by Gram stain and oxidase test to identify *Neisseria sp.* To confirm identification of *N. meningitidis*, *sodC* Syber qPCR for the gene in *N. meningitidis* with melt-curve analysis and MALDI-TOF were used. Of the oxidase positive isolates, 5.5% were confirmed as *N. meningitidis* (5.5%). The other species identified were *Kingella dentrificans*, *Rothia sp, Capnocytophaga sputigena*, *Moraxella catharrhalis* and *Neisseria. lactamica*. There was a 100% congruence of the PCR for *sodC* and MALDI-TOF assignments indicating that the molecular test was highly specific for cultured organisms.

335

The Sequence of the Mature Region of Secreted Proteins Impacts Signal Peptidase 1 Processing

Joanna E Musik[1], Yaramah M Zalucki[1], Christopher J Day[1], Michael P Jennings[1]

1. Griffith University, Gold Coast, QLD, Australia

Bacterial proteins destined for export from the cytoplasm often contain an N-terminal signal peptide. This short (20-30 amino acids) sequence is cleaved by a signal peptidase upon translocation to the periplasm. The sequence requirements for signal peptides have been extensively studied. Less well understood are sequence requirements on the mature protein side that are important for signal peptide cleavage. A recent finding reported a statistical bias against aromatic residues in the second position after the cleavage site (P2') in the mature region of signal peptidase 1(LepB)-dependent secreted proteins. To determine the reason for this bias, the P2' residue was changed to each of the three possible aromatic amino acids in maltose binding protein (MBP); a key model system of protein secretion. The wild-type and aromatic variants were purified and underwent protein staining and Western analysis to see if an increase in the precursor protein could be observed. In comparison to the wild-type, the aromatic P2' MBP variants showed greater precursor accumulation on the protein stains. Surface Plasmon Resonance (SPR) using synthetic peptides encompassing the cleavage site region was used to assess the impact of these mutations on recognition by LepB. SPR analysis showed the P2' aromatic species had similar on-rates, but slower off-rates in comparison to the wild-type. This decreased off-rate was also shown to be able to inhibit the binding of the wild-type peptide to LepB. Therefore, aromatic amino acids present at P2' have an effect on the processing ability of signal peptidase 1. Mature protein residues play a role in signal peptidase processing, and their incorporation in signal sequence prediction software will improve bioinformatics analysis of bacterial secretomes.

336

Ins and outs of blooming *Escherichia coli*

Buddhie S. Nanayakkara[1], David M. Gordon[1]

1. The Australian National University, Canberra, ACT, Australia

Escherichia coli is widely used as an indicator of faecal contamination of drinking and recreational waters. There is a growing body of evidence demonstrating that some *E. coli* strains become naturalized to their secondary habitat: soil, water and sediments. Consequently the presence of *E. coli* in water may not always indicate recent faecal contamination. *E. coli* strains responsible for significantly elevated counts (10,000 – 100,000 cells/100 ml of water) have been reported from fresh water reservoirs across Australia. Multiple strains belonging to phylogenetic groups A and B1 are responsible for these elevated counts, and all possess a Group I capsule originating from *Klebsiella*.

Phylogroup A strains represent the majority of bloom strains. Pan genome analysis based on whole genome sequence data for 330 strains revealed that the ferric citrate uptake system (*fecIRABCDE*) was over-represented among phylogroup A encapsulated strains. The growth rate of strains of four capsule and *fec* operon genotype combinations; [cap+fec+], [cap+fec-], [cap-fec+] and [cap-fec-] in media containing iron and/or citrate was determined. Growth rates were also estimated using carbon sources that differed in their uptake mechanism, and at low (0.01 mM) and high (10 mM) glucose concentrations. The duration of the lag phase was also measured.

Encapsulated strains had a statistically significant growth rate advantage over non-encapsulated strains at low glucose concentrations, irrespective of media composition. The effect of *fecIRABCDE* operon was variable. The strains grew better in trehalose and maltose due possibly to enhanced outer membrane uptake. Lag time of encapsulated strains was significantly shorter than non-encapsulated strains.

The capsule is known to provide protection against adverse environmental conditions and predation, thus enhancing cell persistence in the environment. The experimental results show that encapsulated strains have a shorter lag phase and higher growth rates than the non-encapsulated strains. Thus the enhanced persistence of encapsulated strains means that they are more likely to be present during nutrient influx events, and are also able to outcompete the co-occurring non-encapsulated strains.

337

Extracytoplasmic Methionine sulfoxide reductase protects *Haemophilus influenzae* from oxidative stress caused by HOCl.

Marufa Nasreen[1], Rebeb Dhouib[1], Alastair G McEwan[1], Ulrike Kappler[1]

1. University of Queensland, St. Lucia, QUEENSLAND, Australia

Non-typeable *Haemophilus influenzae* (NTHi) is a host-adapted human pathogen that causes diseases of the respiratory tract such as chronic bronchitis and COPD as well as otitis media. The cytoplasmic defense strategies in NTHi to deal with host-induced oxidative stress are well studied, but little is known about oxidative stress defenses in the periplasm.This project investigates the role of peptide-methionine sulfoxide reductase (Msr), which in NTHi is a fusion of MsrA and MsrB domains, in oxidative stress defense. We characterized the effects of an *msrAB* gene knockout on NTHi physiology and virulence and examined the reductase activity of MsrAB.The *msrAB* gene is co-transcribed with genes encoding a thioredoxin and a protein related to the cytochrome-*c*-type-biogenesis protein CcdA which are likely involved in electron transfer to MsrAB enzyme. Expression of *msrAB* in Hi2019^{WT} increased 45-fold following HOCl challenge, following which the MsrAB protein was present in the periplasmic space and also associated with the outer membrane. An assay for the reductase activity of periplasmic-MsrAB was developed, revealing 0.712U/mg in the presence of methyl-*p*-tolyl-sulfoxide as substrate.We created a mutation in *msrAB* by inserting a kanamycin cassette which was non-polar. Hi2019$^{\Delta msrAB}$ had no growth phenotype *in vitro* and showed no difference in its ability to use different sources of carbon, nitrogen, or sulfur, or in responses to pH change and osmolytes in phenotypic microarrays. Hi2019$^{\Delta msrAB}$ consistently produced 15% and 17% less biofilm under aerobic and microaerophilic conditions, ($p \leq 0.0001$). However, exposure of Hi2019$^{\Delta msrAB}$ to 150mM HOCl resulted in 50% less viable cells compared to the WT and this was also observed when Hi2019$^{\Delta msrAB}$ was exposed to HOCl-producing neutrophils. We propose that Hi-MsrAB is an important enzyme for protecting NTHi against extracellular HOCl stress, a condition that is well described to exist at sites of acute infection and that a secondary role may be in mediating successful host-interactions, especially with neutrophils.

338

Genes involved in protozoan grazing resistance of *Vibrio Vulnificus*

Parisa Noorian[1, 2], Shuyang Sun[2], Diane McDougald[2, 3]

1. BABs, University of New South Wales, Kensington, NSW, Australia

2. ithree Institute, University of Technology Sydney, Sydney, NSW, Australia

3. Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore

Vibrio vulnificus is an autochthonous inhabitant of coastal marine environments where predation by protozoa is a shaping force in the evolution of antiprotozoal mechanisms. These protective mechanisms may also function as virulence factors in animal and human hosts. This study investigates the grazing resistance mechanisms expressed by *V. vulnificus* against protozoa and the potential role of these antiprotozoal factors in providing fitness in the environment.

The resistance to predation by *Tetrahymena pyriformis* of clinical and environmental isolates of *V. vulnificus* with different genotypes was evaluated. The resistance of planktonic cells of *V. vulnificus* isolates to predation by *T. pyriformis* was investigated by determining the numbers of planktonic cells after exposure to *T. pyriformis.* Data shows that place of isolation or genotype did not correlate with grazing resistance of *V. vulnificus* isolates. However, an oyster isolate, *V. vulnificus* Env1, showed significant grazing resistance and toxicity towards *T. pyriformis*.

The whole genome sequence of Env1 was completed, annotated and compared to grazing sensitive strains to identify Env1 unique features that may contribute to grazing resistance. These unique genes include a putative internalin, a putative rearrangement hotspot toxin (rhs), an ankyrin protein, a type 1 secretion system-associated agglutinin RTX membrane protein and a suppressor for copper-sensitivity ScsD. In order to assess the role of these genes in grazing resistance, site-directed mutagenesis was performed and mutants were exposed to predation by different protozoan models. We will discuss the role of each of these genes in predation resistance of *V. vulnificus.*

339

Forward genetics of the human pathobiont *Bacteroides vulgatus* reveals a novel mode of haem-iron acquisition.

Hayley Reed[1], Jakob Begun[2], Mark Morrison[1], Paraic O Cuiv[1]

1. The University of Queensland Diamantina Institute, Translational Research Institute, University of Queensland, Woolloongabba, QLD

2. Mater Research Institute-The University of Queensland, Woolloongabba, QLD, Australia

Inflammatory Bowel Disease (IBD) is a chronic disease characterised by episodic and disabling inflammation of the gut. The gut microbiota is central to the pathogenesis of IBD and consistent with this the onset of active disease is associated with an innate immune response that includes the sequestration of iron to suppress microbial growth. However, with the notable exception of the Proteobacteria, little is known about how this "nutritional immunity" affects the growth of fastidious gut bacteria and their ability to persist in the gut. The human gut bacterium *Bacteroides vulgatus* is widely considered to be a pathobiont – a symbiont that can cause pathology in response to host and/or environmental triggers – and it has been repeatedly implicated in the pathogenesis of IBD. Using a custom high-throughput anaerobic culturing platform we determined *B. vulgatus* has an absolute nutritional requirement for iron and can utilise a wide range of host derived iron sources including haemin, haemoglobin, transferrin, lactoferrin and ferritin to support growth under low iron conditions. *B. vulgatus* is genetically recalcitrant and we thus developed an efficient mutagenesis strategy that exploits the promiscuous RP4

conjugative transfer system and the mariner transposon. We recovered >39,000 mutants and achieved a theoretical coverage of >99% of the genome. Following penicillin-based enrichment to select for *B. vulgatus* mutants defective in haemin utilization we identified no less than three distinct genetic loci that underpin haem iron utilisation. Two of these loci encoded the TonB dependent outer membrane receptor and TonB-ExbBD energy transduction system respectively, while the third had not previously been implicated in haem iron utilisation. We analysed previously published INSeq mutagenesis data for *Bacteroides thetaiotaomicron* and *Bacteroides ovatus* and determined these loci are necessary for optimal fitness during *in vitro* and *in vivo* growth. Our study has provided the first mechanistic insight into iron acquisition by *B. vulgatus*. We contend that genetic dissections offer an approach complementary to genomics to elucidate the functional capacity of fastidious gut bacteria.

340

Identification of novel antimicrobial compounds from Australian Myrtaceae species

Malin M Olsson[1], Peter R Brooks[2], Roy M Robins-Browne[3], Steven M Ogbourne[2], Stephen J Trueman[2], Ji Yang[3], David J McMillan[1]

1. School of Health and Sports Sciences, University of the Sunshine Coast, Maroochydore, Queensland , Australia

2. Faculty of Science, Health, Engineering and Education, University of the Sunshine Coast, Maroochydore, Queensland, Australia

3. Peter Doherty Institute, University of Melbourne, Parkville, Victoria, Australia

Microbial resistance to antibiotics is an escalating problem world-wide with an enormous impact on human health that have led to an urgent need for new antibiotics. The situation is exacerbated by bacteria resistant to multiple antibiotic classes, which causes infections that are very difficult to treat. In particular, the 'ESKAPE' pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acintobacter baumannii, Pseudomonas aureginosa* and *Enterobacter spp.*) have become notoriously problematic by gaining multi-drug resistance and becoming more virulent. The emergence of multi-drug resistant bacteria have led to a renewed interest in drug discovery from natural sources including plants.

Medicinal plants have been used for thousands of years for their antiseptic qualities. The plants produce a vast array of structurally diverse secondary metabolites, many of which have been shown to have antimicrobial activity. However, plants are still a largely untapped biological resource. It has been estimated that only 15% of higher plant species have been phyto-chemically analysed, and even a smaller percentage evaluated for their antibacterial properties.

The major aim of this study is to identify and characterise novel compounds in Australian Myrtaceae species (*Eucalyptus, Corymbia* and *Angophora*) that have antimicrobial activity against multiple ESKAPE pathogens.

Three distinct bioactivity assays will be used to assess antimicrobial activity in extracts from these species. Firstly, standard high-throughput MIC/MBC assays will be used to evaluate bactericidal and bacteriostatic activity. Secondly, biofilm specific screens will be used to determine if extracts inhibit biofilm formation of selected species. Thirdly, we will use a reporter-gene assay to identify inhibitors of the RegA response regulator of *Citrobacter rodentium.* Response regulators are interesting antimicrobial targets because the inhibition of key virulence regulators may attenuate the pathogenicity of the target bacteria while leaving the commensal microflora intact. Selected fractions with bioactivity will be further purified, and pure compounds subjected to additional functional assays.

341

Squirrel gliders in the pasteurellosis spotlight

Lida Omaleki[1], Thotsapol Thomrongsuwannakij[2], Pat J. Blackall[1], Brian M. Forde[3], Scott A. Beatson[3], Nicky B. Buller[4], Sam D. Hair[4], Simone D. Vitali[5], Alisa M. Wallace[5], Conny Turni[1]

1. Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, Queensland, Australia

2. Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University , Bangkok, Thailand

3. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, Queensland, Australia

4. Animal Pathology, Diagnostic Laboratory Services, Sustainability and Biosecurity, Department of Primary Industries and Regional Development, South Perth, Western Australia, Australia

5. Veterinary Department, Perth Zoo, South Perth, Western Australia, Australia

A septicaemic disease outbreak in one of the enclosures at a zoo in Western Australia (zoo A) resulted in the death of three resident squirrel gliders within a month following the introduction of two new gliders from another zoo (zoo B) into the enclosure. Tissue samples from two of the dead gliders as well as oral swabs from the two introduced gliders and a resident woylie confirmed *Pasteurella multocida* as the bacterial cause of the outbreak and also the presence of the organism in the remaining animals. Oral swabs were then taken from thirteen marsupials (10 squirrel gliders two rufous bettongs and one woylie) from four different enclosures at zoo B (enclosures B1 to B4). *P. multocida* was isolated from five of the seven marsupials in enclosure B1, the enclosure where the two introduced gliders came from, as well as residents of enclosures B3 and B4, but not from those cohabiting enclosure B2. The isolates were then analysed via lipopolysaccharide typing, repetitive extragenic palindromic polymerase chain reaction (rep-PCR) typing, multilocus sequence typing (MLST), whole genome sequencing and phylogenomic analysis to investigate isolates relatedness.

The outbreak isolates displayed the same rep-PCR profile, MLST and LPS structure as those obtained from squirrel gliders moved to zoo A, and also those obtained from marsupials at zoo B. Core genome SNP tree demonstrated that the outbreak isolate and those from marsupials at zoo B were clonal, suggesting zoo B as the source of the outbreak. However, the *P. multocida* isolate obtained from the woylie cohabiting with the dead gliders at zoo A was unrelated to the outbreak isolates. Overall, the use of WGS technique has allowed a comprehensive tracking of this disease outbreak and will guide future quarantine and movement protocols.

342

The role of zinc acquisition and zinc tolerance in Group A streptococcal infection

Cheryl-Lynn Y. Ong[1], Mark J Walker[1], Alastair G McEwan[1]

1. School of Chemistry and Molecular Biosciences, Australian Infectious Diseases Research Centre, The University of Queensland, St Lucia, QLD, Australia

Zinc plays an important role in host innate immune function. However, the innate immune system also utilizes zinc starvation ('nutritional immunity') to combat infections. Here, we investigate the role of zinc import and export in protection of *Streptococcus pyogenes* (Group A *Streptococcus*; GAS), a Gram-positive bacterial pathogen responsible for a wide spectrum of human diseases, against challenge from host innate immune defence. In order to determine the role of GAS zinc import and export during infection, we utilized the zinc import (Δ*adcA/AII*) and export (Δ*czcD*) deletion mutants in competition with wild-type in both *in vitro* and *in vivo* virulence models. We demonstrate that nutritional immunity is deployed extracellularly while zinc toxicity is utilized upon phagocytosis of GAS by neutrophils. We also show that lysosomes and azurophilic granules in neutrophils contain zinc stores for use against intracellular pathogens.

344

Root microbial Dynamics influenced by Quorum-Sensing Signal Molecules on Continuous Cropping Soils of Ginseng

Chang Eon Park[1], YeonGyun Jung[1], Huy Pham Quang[1], Jae-Ho Shin[1]

1. School of Applied Biosciences, Kyungpook National University, Daegu, Republic of Korea

Ginseng (*Panax ginseng* C.A Meyer) is traditionally well known as a widely used medicinal product. Since the 1900s, the demand for ginseng has soared, and the plant has been cultivated in earnest. Currently, about 80,000 tons of ginseng are produced worldwide. However, ginseng requires long-term cultivation period of 5 ~ 7 years, and it is necessary to search for a way to reduce extreme damage. Although the series damage is known to be caused by fungi, there have been reports to indirectly deduce the microorganism by isolating the fungi, but the study on the microbial composition of the whole is insufficient.

Quorum-sensing (QS) signaling materials are well known for regulating bacterial cell density. Among them, *N*-acyl homoserine lactones (AHL) is known to control the cell density of Gram-negative bacteria.

In this study, continuous ginseng cultivated sites were located in the Yeongju-si, Republic of Korea. The soil samples were collected with 5 years over continuous cropping history. In addition, we observed that the QS signaling (QSS) molecules regulate bacterial cell density, and we tried to cultivate ginseng in the diseased soil and treat the QSS molecules to change the microorganism. The disease did not occur in the place where the QSS molecules were treated, and the disease occurred in the treatment field in which the soil was sterilized and only treated with water.

Microbiome was analyzed using the next generation sequencing instrument. The V4-V5 region and ITS2 region were used for bacteria and fungi community analysis. The SILVA database and the UNITE database were used for microbiome analysis. In the case of fungi, the results were not well studied globally so that the results obtained using NCBI blastn were more than 97% homologous to establish its own database. As a result, Acidobacteria and Gemmatimonadetes were relatively frequent in bacterial community. In addition, the fungal community showed no tendency in the dominance of *Fusarium* spp.. *Botrytis* fungi were also detected according to the results of the newly constructed database, and it was observed that *Botrytis* fungi were present in the diseased areas.

Biodiversity was analyzed with Shannon-Wiever index, Margalef's richness, and Pielou's evenness. The changes in fungal community were analyzed by the diversity index because there was no particular tendency. The total diversity results showed the bacterial total diversity was decreased in the healthy soils and fungal total diversity was increased in the healthy soils.

1. Li, Y., Ying, Y. and Ding, W. 2014. Dynamics of Panax ginseng rhizospheric soil microbial community and their metabolic function. Evidence-Based Complementary and Alternative Medicine 2014.
2. Miller, M. B. and Bassler, B. L. 2001. Quorum sensing in bacteria. Annual Reviews in Microbiology 55(1): 165-199.
3. Pielou, E. 1975. Ecology diversity. J. Wiley and Sons, New York.

345

The Prevalence of Inhibitory Antibodies in an Australian Cystic Fibrosis Cohort

Amy Pham[1], Daniel Smith [2], Scott Bell[2], Timothy Wells[1]

1. University of Queensland Diamantina Institute , Brisbane, QLD, Australia

2. Adult CF Centre, Prince Charles Hospital, Brisbane, QLD, Australia

Cystic fibrosis (CF) is one of the most prevalent autosomal recessive condition to affect Caucasian populations. It is a multi-system disease which results in a myriad of medical complications for the patient. The difficulties associated with CF often is due to chronic pulmonary infections with *Pseudomonas aeruginosa*. Recently, impaired serum-mediated killing of *P. aeruginosa* was described in patients with bronchiectasis. IgG2 specific to the O-antigen side chains of LPS was identified as the inhibitory factor in serum. The 'inhibitory antibodies' were present in 20% of patients chronically infected with *P. aeruginosa*. Importantly, the presence of inhibitory antibodies correlated with increased respiratory infections and disease severity, evident by respiratory function. It is proposed IgG2 exerts its role as an inhibitor in serum by binding to O-antigen to create a physical blockage, preventing the access of protective antibodies and the membrane attack complex. Here, we investigated the presence of inhibitory antibodies in a new CF cohort, complementing the findings of Wells *et al.,* 2014, and expanding the scale of impaired serum killing to other chronic lung diseases. We established our CF cohort predominately had O-antigen specific antibody to *P. aeruginosa* serotypes, O1, O3, O6 and O11. Further, we found that over ~34% (26/75) of patients had

antibody which impaired bactericidal activity of sera. Interestingly, titre alone was not sufficient to predict serum inhibition, with affinity of antibodies also important. 2/25 patients with impaired killing did not produce high IgG2 titres to O-antigen, yet could still inhibit. Here, O-antigen specific IgA was the inhibitory factor in impaired serum. These findings have improved our understanding of the scope, mechanism and mode of inhibitory antibodies. Importantly, this will aid in the development of early diagnostics and lead to the improvement of targeted immunotherapies.

1. Wells, T.J., D. Whitters, Y.R. Sevastsyanovich, J.N. Heath, J. Pravin, M. Goodall, D.F. Browning, M.K. O'Shea, A. Cranston, A. De Soyza, A.F. Cunningham, C.A. MacLennan, I.R. Henderson & R.A. Stockley, (2014) Increased severity of respiratory infections associated with elevated anti-LPS IgG2 which inhibits serum bactericidal killing. J Exp Med 211: 1893-1904.

346

Regulation of c-di-AMP levels during osmotic stress in bacteria.

Huong Pham[1], Nguyen Nhiep[1], Thu Vu[1], Esteban Marcellin[1], Raquel Lo[1], Nidhi Bansal[1], Liang Zhao-Xun[2], Mark Turner[1]

1. The University of Queensland, ST LUCIA, QLD, Australia

2. School of Biological Sciences, Nanyang Technological University, Singapore

Nucleotide signalling pathways in bacteria are key regulators of cellular responses under external stimuli. Cyclic di-adenosine monophosphate (c-di-AMP) is one of recently discovered messengers in a broad range of bacteria that regulates numerous important cellular processes, however, signals that trigger its accumulation or depletion are poorly understood. Levels of c-di-AMP have been found to inversely correlate with bacterial osmoresistance due to inhibition of potassium and compatible solute uptake systems. In this study, a genetic screen revealed several gain-of-function suppressor mutations in a Kup family potassium transporter that restored osmoresistance in a high c-di-AMP phosphodiesterase *Lactococcus lactis* mutant. Surprisingly these mutations led to significantly elevated c-di-AMP levels. Constitutive potassium uptake was found to trigger c-di-AMP accumulation, possibly in response to increased turgor pressure. In agreement with this, c-di-AMP levels in resting and energised *Lactococcus*, *Lactobacillus*, *Staphylococcus* and *Listeria* were found to be rapidly responsive to external osmolarity changes. These results demonstrate a feedback loop exists whereby c-di-AMP regulates osmoresistance and is in turn modulated by cellular osmotic perturbations in bacteria.

347

The role of phase-variable epigenetic gene regulation in *Streptococcus pneumoniae* pathobiology and vaccine development

Zachary N Phillips[1], Claudia Trappetti[2], James C Paton[2], Michael P Jennings[1], John M Atack[1]

1. Institute for Glycomics, Griffith University, Gold Coast, QLD, Australia

2. Research Centre for Infectious Diseases, University of Adelaide, Adelaide, SA, Australia

Streptococcus pneumoniae is the most common cause of bacterial illness worldwide, causing more than 1.5 million deaths anually. *S. pneumoniae* commonly colonises the nasopharynx of healthy individuals asymptomatically. Despite being heavily studied a complete understanding of the mechanism(s) involved in pneumococcal pathogenesis is lacking. Antibiotic resistance is becoming a major barrier in effective treatment. Current vaccines against *S. pneumoniae* (PCV-13 and PPSV-23) remain ineffective against untargeted strains. A complete understanding of the pathobiology of *S. pneumoniae* will aid development of more effective vaccines and treatments. Our recent work reports a novel, randomly switching, N^6-adenosine DNA methyltransferase (the SpnD39III system) producing six different specificities (alleles A-F). These variants produce six (epigenetically regulated) phenotypes; the first description of a phasevarion (phase-variable regulon) in a Gram-positive organism. Previously reported phasevarions regulate genes mediating immune-evasion, playing key roles in virulence. However, gene expression changes mediated by the SpnD39III system have only been studied in four of the six alleles (A-D), under *in vitro* conditions, and the role of phasevarion switching in colonisation, carriage, and disease has never been investigated. This project aims to characterize the phenotypes of the six SpnD39III alleles, including survival in human blood, biofilm formation, adherence to human cells, and capsule production. The impact on clinically relevant traits will also be analysed: the role of SpnD39III phase-variation in vaccine candidate expression will be investigated, as will response to antibiotics (minimum inhibitory concentrations; MICs). We will also investigate the gene/protein expression profiles of all six allelic variants of the SpnD39III system (alleles A-F), complementing and enhancing our existing data. This will provide a more robust understanding of gene regulation and pathobiology in a pathogen responsible for significant global morbidity and mortality. Findings will direct and inform future vaccine development by identifying the stably expressed antigen repertoire of *S. pneumoniae*.

348

Molecular prediction of the O157:H-negative phenotype prevalent in Australian STEC patients

Alexander P Pintara[1, 2], Christine JD Guglielmino[1], Irani U Rathnayake[1], Flavia Huygens[2], Amy V Jennison[1]

1. Public Health Microbiology, Forensic and Scientific Services, Brisbane, Queensland, Australia

2. Faculty of Health, Queensland University of Technology, Brisbane, Queensland, Australia

Shiga toxin-producing Escherichia coli (STEC) is a food-borne pathogen, where serotype O157:H7 STEC is typically associated with severe disease that can cause life-threatening illness such as haemolytic uremic syndrome. Australia is unique in its STEC epidemiology however, as severe cases are typically associated with non-O157 serogroups, and locally acquired O157 isolates are H-negative/nonmotile. The H-negative phenotype and reduced severity of disease compared to that associated with H7/motile strains are distinct features of Australian O157 strains, where the molecular mechanism behind this phenotype has not been reported. Accurate characterization of the H-negative phenotype is important in epidemiological surveillance of STEC as accurate molecular characterisation of pathogens is necessary to effectively monitor trends in infections and to identify and track sources of outbreaks, so as to enable a rapid public health response. Serotyping is moving away from phenotype-based methods, as next generation sequencing allows rapid extrapolation of serotype through *in silico* detection of the O-antigen processing genes, *wzx*, *wzy*, *wzm*, and *wzt*, and the H-antigen gene, *fliC*. The detection and genotyping of *fliC* alone is unable to determine the motility of the strain as most Australian O157:H-negative strains carry an H7 genotype yet phenotypically are nonmotile; thus, many are mischaracterised as H7 strains by *in silico* serotyping tools. Comparative genomic analysis of flagellar genes between Australian and international isolates was performed and an insertion at nucleotide (nt) 125 in the *flgF* gene was identified in H-negative isolates. Chi-square results showed that this insertion was significantly associated with the H-negative phenotype ($P < 0.0001$). Phylogenetic analysis was also completed and showed that the Australian H-negative isolates with the insertion in *flgF* represent a clade within the O157 serogroup, distinct from O157:H7 serotypes. This study provides a genetic target for inferring the nonmotile phenotype of Australian O157 STEC, which increases the predictive value of *in silico* serotyping.

349

Accumulation of Total Lipids and Triacylglycerides as Biodiesel Precursors upon Co-culturing a Phycospheric Bacterium with Indigenous *Chlorella vulgaris*

Katherine L. Pintor[1], Pierangeli G. Vital[1], Metha Meetam[2]

1. Institute of Biology, University of the Philippines, Quezon City, Metro Manila, Philippines

2. Faculty of Science, Mahidol University, Ratchathewi, Thailand

The cultivation of microalgae for the production of biomass and associated valuable compounds such as neutral lipids in the form of triacyglycerides (TAGs) has gained growing interest over the years. Currently, there has been a substantial attention given to *Chlorella*species as source of feedstock for biodiesel production due to its ability to grow rapidly, and accumulate high amounts of TAGs stored in the cytosolic lipid bodies. However, when grown under favorable conditions, *Chlorella*does not accumulate neutral lipids. This has become the major bottleneck in meeting standard industrial requirements for biofuels. To address this drawback, the study utilized a new approach by engineering a microalgal-bacterial community in the phycosphere. Hence, themain objective of this project is to determine the influence of a previously isolated phycospheric bacterium *Rhizobium*sp. to axenic and xenic freshwater *C. vulgaris*'total lipid content and TAG productivity determined by gravimetric quantification, TLC, and GC-MS. Results showed that xenic *C. vulgaris*inoculated with *Rhizobium*(CV+B) displayed more than two-fold increase on both total lipids (23.87 ± 1.23) and TAG productivity (8.21 ± 2.07)in comparison to the control and other variables. Meanwhile,the extracted TAGs from axenic *C. vulgaris*(CA)exhibited a variety in its FA composition (C16, C18, C18:1) amounting to 11.53%, 10.95% and 6.52%, respectively. Our study has demonstrated an efficient means of inducing microalgal biodiesel precursors by co-cultivation of algae and bacteria. To our knowledge, this is the first research undertaking in the Philippines that revealed its potential application for biofuel production.

350

Evaluating the Extensively Drug-Resistant *Klebsiella pneumoniae* Resistome via MinION Sequencing

Miranda E Pitt[1], Son Hoang Nguyen[1], Tânia PS Duarte[1], Mark AT Blaskovich[1], Matthew A Cooper[1], Lachlan JM Coin[1]

1. Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland, Australia

Introduction

Klebsiella pneumoniae is one of the leading causes of nosocomial infections, frequently possesses multidrug resistance and subsequently results in high mortality[1]. Utilising sequencing as a rapid diagnostic for bacterial infections has advanced significantly, in particular, MinION sequencing (Oxford Nanopore Technologies)[2]. This portable device is capable of real-time analysis and reading long fragments of DNA and RNA. This study sequenced four extensively drug-resistant (XDR) *K. pneumoniae* clinical isolates in order to assemble these genomes, discern the differential expression of resistance genes and ascertain the time required for detection.

Methods

Isolates were obtained from the Hygeia General Hospital (Greece)[3]. DNA and RNA were extracted from a paired inoculum and long fragment DNA was acquired using the MagAttract HMW DNA kit. RNA underwent an mRNA enrichment, poly(A) ligation and direct RNA sequencing on MinION R9.4 flowcells. The real-time emulation was conducted as previously described[4].

Results

DNA sequencing identified the majority of acquired resistance (≥75%) resided on up to 5 plasmids in these isolates. The real-time emulation detected ≥70% of resistance genes in 2 hours for all isolates. Direct RNA sequencing successfully revealed aminoglycoside, beta-lactam, trimethoprim and sulphonamide resistance within 6 hours. In several instances, quinolone, rifampicin and phenicol resistance was apparent, although dependent on the level of transcription. Fosfomycin, macrolide and tetracycline resistance was absent, however, these genes were validated to have low expression via qRT-PCR.

Conclusion

MinION sequencing was capable of detecting antibiotic resistance in these XDR *K. pneumoniae* isolates within hours and differential expression of these genes was successfully validated via direct RNA sequencing.

1. Martin RM, Bachman MA. 2018. Front Cell Infect Microbiol. 8:4.

2. Gardy JL, Loman NJ. 2018. Nat Rev Genet. 19:9-20.

3. Pitt ME, Elliott AG, Cao MD, Ganesamoorthy D, Karaiskos I, Giamarellou H, Abboud CS, Blaskovich MAT, Cooper MA, Coin LJM. 2018. Microb Genom. doi: 10.1099/mgen.0.000158.

4. Cao MD, Ganesamoorthy D, Elliott AG, Zhang H, Cooper MA, Coin LJ. 2016. Gigascience. 5:32.

351

Resistance for Dummies

april pottinger[1]

1. Microbiology, Royal Hobart Hospital, Hobart, TAS, Australia

Bacterial resistance is the capacity of bacteria to withstand the effects of antibiotics or biocides that are intended to kill or control them. There are three fundamental mechanisms of antimicrobial resistance; (1) enzymatic degradation of antibacterial drugs that inactivate the drug, (2) alteration of target bacterial proteins so the drug no longer recognizes it, and (3) changes in membrane permeability to antibiotics leading to a decreased intracellular concentration of antibiotics.

Since the first introduction of penicillin in the 1920's, there have been numerous new antimicrobials produced in response to increasing resistance of bacteria. The outcome however has always been the same; organisms evolve and find ways to resist these antimicrobials.

Resistance among gram negative bacilli is becoming increasingly common and plays an important role in laboratory work. Enterobacteriaciae can exhibit all three types of resistance mechanisms. The most commonly seen worldwide is beta-lactam resistance, which involves enzymatic degradation of antibacterial drugs. This is carried out by beta lactamases, which are enzymes that inactivate beta-lactam antibiotics by hydrolysis. There are two schemes used to classify beta-lactamases, the Ambler and the Bush-Jacoby-Medeiros. Within these schemes, there are three categories of beta-lactam resistance, that being carbapenemase resistant enterobacteriacae (CPE), AmpC resistance and extended spectrum beta lactamases (ESBL'S). There are numerous steps in the laboratory to identify these resistance genes, involving specialised susceptibility testing, Etests, PCR and rapid screening tests. These are critical to properly guide antibiotic therapy for patients, implement appropriate isolation measures, and follow any emerging resistance patterns around the globe.

352

Investigation into resistance acquisition mechanisms in methicillin-resistant *Staphylococcus aureus* (MRSA) by means of resistance induction and next-generation sequencing

Anggia Prasetyoputri[1], Miranda E Pitt[1], Minh Duc Cao[1], Soumya Ramu[1], Angela Kavanagh[1], Devika Ganesamoorthy[1], Arnold Bainomugisa[1], Matt A Cooper[1], Lachlan Coin[1], Mark AT Blaskovich[1]

1. The University of Queensland, St Lucia, QLD, Australia

The burden of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is exacerbated by an increasing prevalence of vancomycin-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA)[1], including emerging resistance to alternative antibiotics such as linezolid and daptomycin[2]. The mechanisms by which MRSA acquires antibiotic resistance in the clinic, however, are not fully understood. We have recently created a series of new semi-synthetic glycopeptide analogues designed to overcome resistance[3]. Utilizing *in vitro* resistance induction assays will not only allow us to discern the resistance acquisition in MRSA, but also to unravel the potential modes of action for novel glycopeptides. To do so, MRSA strain (ATCC43300 – vancomycin MIC: 1 µg/mL) was passaged for 20 days in the presence of increasing sub-lethal concentrations of five clinically used antibiotics (vancomycin, daptomycin, dalbavancin, linezolid and cefepime) and three novel next-generation glycopeptide analogues. Day 20 replicates (n=8 per treatment group) were sequenced (Illumina NextSeq) and variants determined via GATK analysis. The results showed that the majority of day 20 replicates exposed to clinically used antibiotics developed a resistant phenotype (MIC ≥2 µg/mL). In contrast, a slow progression in resistance was observed in replicates exposed to dalbavancin and the novel glycopeptides and their MIC values remained below the clinical breakpoint (MIC ≤0.25 µg/mL). Sequencing revealed previously known mutated genes in clinical isolates including *walK*, *agrC*, and *pbpB* genes associated with reduced vancomycin susceptibility, as well as other genomic variants not previously reported. Additionally, mutated *mprF* and *rpoB* genes associated with daptomycin resistance were found in daptomycin-induced replicates. Replicates exposed to the three novel glycopeptides exhibited additional genomic variants affecting different pathways, suggesting potential multiple modes of action. These results demonstrated that *in vitro* induced resistance in MRSA affected similar genes to those identified in clinical isolates, validating that it can predict clinical resistance. The novel glycopeptides, which retain good potency against MRSA, exhibited mutations in more pathways that suggest multiple modes of action compared to clinically used antibiotics.

1. Blaskovich, M.A.T. et al., (2018). Developments in glycopeptide antibiotics. ACS Infect Dis 4, 715-735.
2. Nannini E. et al., (2010). Resistance or decreased susceptibility to glycopeptides, daptomycin, and linezolid in methicillin-resistant Staphylococcus aureus. Curr Opin Pharmacol 10, 516-521.
3. Blaskovich M.A.T. et al., (2018). Protein-inspired antibiotics active against vancomycin- and daptomycin-resistant bacteria. Nat Commun 9, 22.

353

Mixed *Eucalyptus* and *Acacia* plantations: assessing the link between bacteria community and C-N functions in the soil and litter interface

Arthur Prudêncio de Araujo Pereira[1], Ademir Durrer[1], Thiago Gumiere[2], JunTao Wang[3], José Leonardo Moraes Gonçalves[1], JAY PRAKASH VERMA[4], Brajesh Kumar Singh[5], Elke Jurandy Bran Nogueira Cardoso[1]

1. Luiz de Queiroz College of Agriculture - University of São Paulo, Piracicaba, SãO PAULO, Brazil
2. Institut national de la recherche scientifique, Centre Eau Terre Environnement, Quebec City, Canada
3. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
4. Institute of Environment and Sustainable Development, Banaras Hindu University, Banaras, UP, India
5. Hawkesbury Institute for the Environment, Western Sydney University, Sydney, NSW, Australia

Poster Abstracts

The stimulus to organic matter cycling and, consequently, the increase in carbon (C) and nitrogen (N) pools in the soil are the most important benefits in mixed *Eucalyptus* plantations with N-fixing trees. However, how bacteria community can contribute to a better C and N dynamics remain poorly understood in this type of forest management. Here, we evaluated interactions between bacteria community and C-N functions in soil and litter interface resulting from pure and mixed *Eucalyptus grandis* and *Acacia mangium* plantations. Overall, we hypothesized that bacteria community (diversity, structure, composition and genes abundance) is different and closely related to C-N improvement in that mixed plantations. We sequenced the 16S rRNA gene (V4 region) using 515F and 806R primers into MiSeq Illumina® sequencing platform and assessed the link between bacteria community and C-N functions (i.e, microbial and enzymes activity, functional genes and soil-litter nutrient cycling). Soil and litter samples from *E. grandis* without (E) and with N addition (E+N), a mixed system between *E. grandis* and *A. mangium* (E+A) and a pure *A. mangium* (A) treatments were collected at 27 and 39 months after planting. We found that mixed plantation (E+A) increase bacterial diversity (soil and litter) and gene *nif*H (soil) abundance, while pure *E. grandis* reduced it (especially E+N). Also, we find a strong correlation between *Rhizobium* and *Sphingomonas* with *nif*H and soil N contents (especially at 27 months). The Total-N content may have regulated bacterial structure, mainly at litter interface. Our study provides a novel evidence of the importance of *A. mangium* in a mixed system with *E. grandis* for C-N cycle in sandy soils conditions, with low organic matter content. It is extremely important because can minimize the mineral fertilizers use and improve soil and plant health.

354

Prevalence, Characteristics and behaviour of bacterial dairy contaminants

Behrad Radmehr[1], Enzo Palombo[1], Bita Zaferanloo[1]

1. Department of Chemistry and Biotechnology, Swinburne University Of Technology, Hawthorn, VIC, Australia

Milk and dairy products are considered to be at high risk of bacterial contamination. *Escherichia coli* and *Bacillus cereus* are common contaminants of raw and processed milk and their recovery in milk and dairy products may be a health concern because of the possible presence of pathogenic strains. The present project aims to study the prevalence of *E. coli* and *B. cereus* in raw milk, and their antimicrobial resistance and biofilm formation ability of isolates.

A total 60 samples of raw milk were collected from a Victorian dairy producer between September 2106 and July 2017. Isolation of *E. coli* and *B. cereus* was carried out according to conventional culture methods and confirmed with MALDI-TOF MS. All *E. coli* and *B. cereus* isolates were subjected to antimicrobial susceptibility tests based on the Clinical and Laboratory Standards Institute (CLSI) criteria using the disk diffusion method. Biofilm formation was assessed using the colorimetric microplate method.

E. coli and *B. cereus* were isolated from 90% and 33.3% of raw milk samples, respectively. Antimicrobial susceptibility testing of *E.coli* showed a high prevalence of antibiotic resistance to Erythromycin, Ampicillin, Streptomycin and Tetracycline. Antimicrobial susceptibility testing of *B. cereus* showed a high prevalence of antibiotic resistance toward Ceftriaxone, Penicillin and Ampicillin. All isolates were sensitive to Ciprofloxacin, Clindamycin, Gentamicin, Tetracycline and Vancomycin. According to the results, most tested *E. coli* strains produced biofilm. In contrast, less than 50% of *B. cereus* produced biofilm. The high level of *E. coli* and *B. cereus* contamination coupled with their capacity to produce biofilm can be a concern for dairy producers. The high level of contamination, antimicrobial resistance and biofilm formation ability warrants further investigation of other properties, including genetic diversity and toxigenic potential.

355

Functional analysis of O-antigen modifying enzyme, *O*-acetyltransferase B, of Shigella flexneri

Munazza Rajput[1], Naresh Naresh Verma[1]

1. Australian National University, Canberra, ACT, Australia

Background: Shigellosis, an acute gastrointestinal disease is caused primarily by the bacterium *Shigella flexneri*. Upon ingestion, *S. flexneri* initiates a serotype-specific immune response that targets the O-antigen of pathogen's lipopolysaccharide. O-antigens subunits are modified by the addition of chemical moieties, which give rise to new serotypes of *S. flexneri*. Nineteen different serotypes of *S. flexneri* have been recognized. A recently identified O-antigen modifying gene, *O*-acetyltransferase B *(oacB*), which adds an acetyl residue at either position 3 or 4 of Rhamanose^{III} (3/4-O-acetylation) in serotypes 1a, 1b, 2a, 5a, 7a, Y, and 6 and at position 6 of N- acetylglucosamine (6-O-acetylation) in serotypes 2a, 3a , Y and Yv of the O-antigen subunits. Topologies of other serotype converting proteins like glucosyltransferases (Gtr) and acetyltransferase (Oac) of *S. flexneri* have been solved experimentally whereas the topological analysis of OacB is yet to be determined.

Materials/methods: Topology prediction of OacB was performed using membrane protein prediction programs such as DAS, HMMTOP, TMHMM and TopPred .Identification of conserved critical residues and domains was carried out using side-directed mutagenesis and functionality of the protein was determined by slide agglutination and western blots using specific antibodies to acetyl modification.

Results: Hydrophobicity analysis showed that OacB similar to its close homologue *O*-acetyltransferase (Oac) from serotype 3b is a transmembrane protein with 10 transmembrane segments, 11 loops, and cytoplasmic N and C termini. Bioinformatics analysis revealed that OacB has several motifs. Mutants were created by mutating conserved amino acids within and outside these motifs. Amino acids critical to function were identified via slide agglutination test and western blots using O-acetyl specific antisera. Five mutants showed a negative agglutination reaction hence turned protein nonfunctional.

Conclusions: The importance of a number of amino acid residues within the conserved domain and the homologous region with regard to function of OacB was determined. Identified amino acids may play an important role in forming the catalytic domain within the acetyltransferase homologous region. The study furthers our understanding of the structure, function of a serotype converting protein, and will contribute to vaccine development research against shigellosis.

356

Rapid Detection of Microbes Using DNA-PNA Hairpin Probes and the $DiSC_2(5)$ Dye

Josh T Ravensdale[1, 2], Ranil Coorey[3, 2], Gary A Dykes[1, 2]

1. Department of Health Sciences , Curtin University, Bentley, WA, Australia

2. Curtin Health Innovation Research Foundation, Curtin University, Bentley, WA, Australia

3. School of Molecular and Life Sciences, Curtin University, Bentley, WA, Australia

Rapid identification of pathogenic bacteria on meat processing surfaces is of critical importance to the industry. This project aims to investigate the potential of using the hybridisation chain reaction (HCR) to detect bacteria on different surfaces. The 3 main components of the HCR are 2 hairpin loop oligonucleotide probes and sample DNA. In the presence of a target DNA sequence the hairpin probes will bind to the DNA and form large hybrid complexes. We propose using DNA and peptide nucleic acid (PNA) probes in a buffer containing 3,3 diethylthiadicarbocyanine iodide ($Disc_2(5)$) dye to identify target genetic sequences. If the target sequence is present in a sample, hybridisation will occur and the buffer will change from a blue to a purple colour. This procedure does not rely on enzymatic or antibody-antigen reactions and could become a cheap, quick and highly sensitive tool that can be used in the industry. We have synthesised HCR probes designed to bind to the superoxide dismutase gene (*sodM*) of *Staphylococcus aureus* and the invasion Protein A gene (*invA*) of *Salmonella* Typhimurium. We have found that modifying the sequence length, GC content and including certain sequence patterns in different regions of the probes affects the sensitivity and stringency of the probes when hybridising to positive control "initiator" molecules. As of yet we have not successfully hybridised these probes to the *sodM* or *invA* sequence on bacterial DNA. When we increased the length of the initiator sequences to achieve a 10bp overhang on the 5' and 3' ends after hybridisation, hybridisation efficacy decreased. Using restriction endonucleases to fragment the genes has produced weak positive signals. We are currently re-optimising and re-designing our probes to get a clearer positive signal. Once the proof of concept has been established these probes can be easily modified for a broad range of genetic screening applications including determining carriage of genetic diseases in patients, and developing antimicrobial resistance gene profiles from clinical samples.

357

Pseudomonas stutzeri skin and soft tissue infection with bacteraemia

Alan J Rogers[1]

1. Pathwest LMWA, Murdoch, WA, Australia

The significance of Pseudomonas species other than *P.aeruginosa* from clinical samples is often questionable. Infections are usually associated with immunocompromised state, endovascular prosthetic devices, or are iatrogenic. Although they have low virulence, they survive harsh environments, and can contaminate medical supplies. Tolerance to antiseptic solutions can lead to pseudobacteraemia. *P.stutzeri* is an unusual cause of human infection, with bacteraemia more commonly associated with contaminated dialysis fluid (1).

A 91 year old gentleman was admitted with severe skin and soft tissue infection of his left leg following a laceration whist sailing on the Swan River. He cleaned and dressed the wound himself, using old antiseptic liquid. He developed wound infection and was prescribed cephalexin by his GP with initial improvement, but attended ED with worsening swelling. CRP was 340mg/L with neutrophilia of 13.4 x 10^9/L. He was commenced on IV flucloxacillin. Inflammatory markers remained elevated and vancomycin was added. Two days into admission the aerobic bottles of both sets of blood cultures grew *Pseudomonas stutzeri* (MALDI-TOF score >2.0). A wound swab subsequently had abundant growth of the same, although not seen on gram stain. In light of his failure to improve, after two additional sets of blood cultures were taken antimicrobial therapy was changed to cefepime.

He clinically and biochemically improved following switch to cefepime, and was subsequently changed to oral ciprofloxacin to complete 3 weeks total. The aerobic bottles of both of the second sets grew *P.stutzeri*, confirming true bacteraemia. There was no evidence of immune compromise bar senescence due to age. Old antiseptic solution is a possible source of his infection, as is the environment of his initial injury.

1. 1. Jorgensen JH, Pfaller MA, Carroll KC, Funke G, Landry ML, Richter SS, et al. Manual of Clinical Microbiology, Eleventh Edition: American Society of Microbiology; 2015.

358

Mechanisms of action of plant extracts against *clostridium difficile*

Niloufar Roshan Hesari[1], Thomas Riley[1, 2, 3, 4], Daniel Knight[3], Katherine Hammer[1]

1. School of Biomedical sciences, University of Western Australia, Crawley, WA, Australia

2. School of Medical & Health Sciences, Edith Cowan University, Joondalup, WA, Australia

3. School of Veterinary & Life Sciences, Murdoch University, Murdoch, WA, Australia

4. Microbiology, PathWest, Nedlands, WA, Australia

Backgrounds and aims: *Clostridium difficile* causes disease ranging from self-limiting diarrhoea to severe pseudomembranous colitis. Antimicrobial treatment failures and patients with multiple recurrences have driven the search for new therapies. Some natural products have broad-spectrum antimicrobial activity with several showing activity against *C. difficile*. Understanding the mechanism of action of these products is important to further characterise their efficacy. Thus, this study aimed to investigate the mechanism of action of five natural bactericidal products, cinnamon root powder, peppermint oil, *trans*-cinnamaldehyde, menthol and zingerone and four bacteriostatic products, fresh garlic bulb extract, garlic clove powder, allicin and *Leptospermum* honey against two *C. difficile* strains.

Methods and results: As determined by measuring the optical density at 620 nm, none of the products caused bacteriolysis. The time-kill assay showed a > 3 log_{10} reduction in *C. difficile* viable counts by all five bactericidal products after 24 h of exposure. An ATP-leakage assay showed that all five products at most concentrations significantly reduced the intracellular ATP after 1 h of incubation ($P \leq 0.01$). Alterations in cell permeability were assessed by measuring the leakage of 260-nm absorbing materials, protein leakage using Bradford assay and the propidium iodide uptake assay. All five bactericidal products damaged the cell membrane as seen in two or more cell permeability assays. The effect of three bacteriostatic products on protein synthesis was determined using an *Escherichia coli* S30 extract system, and only *Leptospermum* honey (16% w/v) showed inhibition of prokaryotic protein synthesis ($P < 0.01$). None of the products showed elevated minimum inhibitory concentrations against strains of *C. difficile* harbouring DNA gyrase mutations, or conjugative transposons carrying *ermB* and *tetM*.

Conclusions: The findings indicate that damage to the cytoplasmic membrane may contribute to the mechanism of action of several natural products against *C. difficile*. Also, the absence of cross-over mechanisms of resistance between standard antibiotics and natural products are suggested. Further studies are required to determine the efficacy of these products *in vivo*.

359

Effect of natural products on the production and activity of *Clostridium difficile* toxins *in vitro.*

Niloufar NR Roshan Hesari[1], Thomas TVR Riley[2, 3, 4], Daniel DK Knight[3], Katherine KH Hammer[1]

1. School of Biomedical sciences, University of Western Australia, Crawley, WA, Australia

2. School of Medical & Health Sciences, Edith Cowan University, Joondalup, WA, Australia

3. School of Veterinary & Life Sciences, Murdoch University, Murdoch, WA, Australia

4. Microbiology, PathWest, Nedlands, WA, Australia

Backgrounds: *Clostridium difficile* infection (CDI) a toxin-mediated disease of the colon and remains a major clinical burden to global healthcare systems. *C. difficile* virulence is primarily attributed to the production of large clostridial toxins TcdA (toxin A) and TcdB (toxin B), thus therapeutic strategies which reduce toxin production and activity can significantly decrease the pathogenicity of CDI and improve patient outcomes. This study investigated the effect of a range of natural products on toxin production and activity of *C. difficile in vitro*.

Objective: To investigate the effect of a range of natural products on the production and activity of *C. difficile* toxins *in vitro*.

Methods: Twenty-two natural products were investigated against four *C. difficile* strains. The toxin protection activity of products was determined using Vero and HT-29 cell cytotoxicity and neutral red uptake assays. Indirect effect of products on toxin-mediated cytotoxicity was determined using the same cell lines. Based on the results from toxin activity assays, seven out of the 22 products were selected to determine their effects on toxin production of *C. difficile* using ELISA.

Results: Zingerone (0.3 mg/ml) protected both Vero and HT-29 cells from *C. difficile* cytopathic effects, confirmed by a neutral red uptake assay ($P < 0.05$). The three Western Australian *Leptospermum* honeys (4% w/v), onion juice (12.5% v/v) and *trans*-cinnamaldehyde (0.005% v/v) reduced both toxin production and activity of *C. difficile* significantly ($P \leq 0.023$). Garlic powder (4.7 mg/ml) also showed a significant reduction in toxin activity ($P \leq 0.047$), but had no effect on toxin production.

Conclusions: Five out of 22 natural products reduced both *C. difficile* toxin production and activity *in vitro*. Zingerone showed protection against the cytopathic effect of *C. difficile* toxins likely through blocking either the toxin binding sites on the toxin molecule or the host cell receptors.

Significance and Impact of the Study: This study encourages further investigation of natural products against *C. difficile* toxins *in vivo*.

360

Predicting VP meat quality and spoilage in domestic and international supply chain

Tom Ross, Mandeep Kaur

Food waste is an increasing concern both because of costs to industry productivity and because of the need to assure food security.

Australia is a relatively large producer of red meat (beef and lamb) and exports those products to many nations. The products are usually exported in vacuum-packs (VP), and shipped at low temperatures (~ -1°C). Under these conditions, there is no perceptible loss of quality for up to 160 days for beef products, and ~90 days for lamb products. If, however, temperature control is lost, the quality of the product can deteriorate more rapidly. The ability to quantify the loss of quality or shelf life due to such lapses of temperature control would enable better decisions to be made about the disposition of such products, i.e., rather than simply discarding products for which temperature control has been temporarily lost.

Quality standards for VP meats are often expressed in terms of 'total viable counts' but in VP products TVC is dominated by lactic acid bacteria that do not necessarily cause spoilage.

We studied changes in TVC and organoleptic quality in vacuum packed beef and lamb primals stored at a range of temperatures (-1, 2, 4, 8°C).

From the microbiological and organoleptic assessments we were able to develop a predictive mathematical model for the end of quality shelf life of Australian VP beef and lamb products.

The reliability of the predictive model was evaluated by a series of trials in export supply chains including Japan, and the Middle East and a domestic supply chain of a national retailer.

The results supported the reliability of the model for vacuum-packed beef and lamb products, but also showed that the model was less reliable for product in modified atmosphere packaging, or reprocessed and repackaged in an aerobic overwrap.

The model has been incorporated into a software tool available to Australian meat processors to enable them to better manage their value chains and reduce product waste as well as maintaining the quality reputation of the Australian meat industry.

This poster describes the development of the model, the relationship between microbiological and sensory quality metrics, and the evaluation of the model against independent 'real world' data.

361

Investigating levels of parvovirus B19 DNA and genotypes circulating in Australian blood donors

Eileen Roulis[1], Elise Gorman[1], Robert Flower[1], Helen Faddy[1]

1. Research and Development, Australian Red Cross Blood Service, Kelvin Grove, Queensland, Australia

Parvovirus B19 (primate erythroparvovirus 1 or B19V) is a single stranded DNA virus of the genus erythroparvoviridae. It is a respiratory virus with erythroid precursor cell tropism, presenting frequently in children as “fifth disease of childhood”. The majority of infections in adults are asymptomatic or result in non-specific flu-like symptoms. B19V infection can result in complications in immunocompromised and vulnerable individuals, including pregnant women. Transmission of B19V through blood transfusion is well documented from donations containing a high B19V titre, and a small number of probable cases of transfusion-transmitted B19V have been reported in Australia. Three genotypes of B19V are recognised, with genotype 1 responsible for the majority of infections worldwide. The genotypes circulating in Australia are unknown. We aimed to determine the prevalence of B19V DNA among Australian blood donors, as well as quantify the levels of B19V DNA and investigate genotype/s in B19V DNA positive samples. Plasma samples from 4232 donors were collected nationally, and screened in duplicate for the presence of B19V DNA by real-time polymerase chain reaction (PCR). Samples reactive on at least one replicate were tested with a second PCR at an external reference laboratory. Viral load was determined for positive samples by quantitative PCR assay with reference to the WHO B19V standard. Twelve samples tested initially reactive B19V DNA, of which 10 were confirmed (0.23%). Viral loads in the B19V DNA positive samples varied from log 1.90 IU/mL to log 6.25 IU/mL, with the mean B19V DNA level log 3.26 IU/mL (1.58 x 10^3 IU/mL). Two donations (0.047%) demonstrated a viral load of over 10^5IU/mL, evidence of in 1 in 2116 donations with potential for transfusion-transmission. Next-generation sequencing is currently being undertaken to determine the B19V genotype/s found in Australian blood donors. As the most common B19V genotype worldwide is genotype 1, it is probable that this is also the predominant genotype circulating in Australia.

362

Potential to analyse bacterial contamination of platelets utilizing 16S metagenomics

Lina Rustanti[1], Eileen Roulis[1], Robert Flower[1], Helen Faddy[1]

1. Australian Red Crood Blood Service (ARCBS), Brisbane, QLD, Australia

A risk associated with transfusion of platelet products is transfusion-transmitted bacterial infection as platelet concentrates are stored at 22 ºC with gentle shaking, suitable conditions to promote bacterial growth. In Australia, this risk is minimised by bacterial contamination screening (BCS), however false positive results can result in component recall. Cumulative data in Australia from 2014 to 2017 demonstrated the rate of BCS initially reactive was 6 per 1000 platelet components comprised of confirmed positive 1/1000, false positive 4/1000 and indeterminate (unconfirmed initially reactive samples) 1/1000.

Using next generation sequencing (NGS) targeting the *16S rRNA* gene, we aim to investigate whether BCS indeterminate results are signals resulting from the presence of microorganisms that do not grow under culture conditions or low concentrations of slow growing microorganisms. As a proof of principle, we examined the microbiome in plasma samples from 10 blood donations. DNA extracted from *E.coli* culture and plasma spiked with *Coxiella burnetti* were used as positive controls.

Signals for the *E.coli* and *C.burnetti* DNA were as expected providing evidence that the processing and analytical procedures were suitable for purpose. Amongst the other 10 samples, evidence for low levels of two bacterial genera was found. Using 95% sequence similarity as a threshold, 2 out of the 10 samples were positive for *Stenotrophomonas spp.*, and 1 was positive for *Bosea spp*. These are environmental microorganisms and are unlikely to be human pathogens, although nosocomial infection related to *Stenotrophomonas sp.* has been reported.

These preliminary results demonstrate that *16S rRNA* NGS is a highly sensitive tool for detection of bacterial contamination; however, procedures for validation of low level signals may be required. The application of *16S* metagenomics has the potential to provide rapid identification of bacterial types and raises new questions in relation to policies for platelet BCS, including how to manage units with indeterminate results and those with low levels of environmental organisms that are not detected by BCS.

363

Bacterial communities vary between healing and non-healing diabetic foot ulcers (DFUs) - An update on the DFU microbiome.

Sumeet Sandhu[1], Irani Rathnayake[1], Steven Rudd[1], Flavia Huygens[1]

1. Institute of Health and Biomedical Innovation (IHBI), Queensland University of Technology, Brisbane, Queensland, Australia

Introduction

Diabetic foot ulcers (DFUs) are an increasingly common problem in diabetic patients. . Every three hours, someone in Australia loses a lower limb as a direct result of diabetes-related foot disease. It has been hypothesized that the microbiome of DFUs is a contributing factor in delayed wound healing. Very little is known about the presence and shifts in microorganisms that occur over time that are associated with DFU. This study examines the microbial diversity and abundance associated with DFUs using genome based methods.

Methods

Two diabetic patient cohorts (n=58 patients) with foot ulcers were studied over a period of 12-24 weeks. Genomic DNA (gDNA) was extracted from patient swab samples (n=362) and amplicon libraries were constructed targeting the V1 and V2 region of the prokaryotic 16S rRNA gene. The Ion Torrent Personal Genome Machine (PGM) was used for amplicon sequencing and the bioinformatics pipeline “mothur” was used to perform a quality control and analysis of the sequences obtained. Statistics and data visualisation was performed using Calypso.

Results

Approximately 6,416,041 high-quality sequences, with an average of 29,704 sequences per sample were generated. Sequencing analysis revealed differences in the microbiome composition of healing and non-healing DFUs as the abundance of some bacterial genera was statistically significant ($p<0.05$) in non-healing DFUs and vice versa.

Conclusion

This study suggests that the significant differential abundance of certain bacterial genera in chronic DFUs can be used as biomarkers to inform ulcer outcome and can inform the use of selective and appropriate antimicrobials. A greater understanding of the diabetic foot ulcer microbiome will help guide new treatment strategies to effectively control the infection and promote healing. This will in turn benefit patients suffering from these ulcers and improve their quality of life.

364

Salmonella response to heat and pH in meat juice

Amreeta Sarjit[1], Josh T RAVENSDALE[1], Ranil Coorey[1], Narelle Fegan[2], Gary A Dykes[1]

1. School of Public Health, Curtin University, Bentley, Western Australia, Australia

2. CSIRO Agriculture and Food, Werribee, Australia

Salmonella enterica is a major cause of bacterial gastrointestinal food-borne infection. Survival of, and cross-contamination by, *Salmonella* in red meat processing facilities may lead to human disease. Interventions such as application of hot water during processing may reduce this risk. This study examined the effect of gradual heat treatment to 70°C and heat shock treatment at 70°C for 5 min on *S.* Anatum (2), *S.* Heidelberg (1), *S.* Saintpaul (1) and *S.* Typhimurium (1) in beef, in lamb and in goat meat juice and phosphate buffered saline (PBS).The effect of pH on heat treatment was also examined by altering the pH of the respective meat juices (beef pH 5.9, lamb pH 5.6 and goat pH 5.6) to match that of PBS (pH 7.4) and vice versa. Untreated controls were included in all experiments. Bacterial numbers in all meat juices and PBS were determined on thin layer xylose lysine deoxycholate agar. In lamb juice, all treatments significantly ($p<0.05$) reduced numbers of *Salmonella* (~2.84 – 6.49 log cfu/ml). In goat meat juice most treatments reduced numbers of *Salmonella* below the limit of detection (>7.24 log cfu/ml), except for gradual heat treatment which significantly ($p<0.05$) reduced numbers of *S.* Anatum (~3.31 log cfu/ml) at pH 5.6. In beef juice gradual heat treatment significantly ($p<0.05$) reduced numbers of *Salmonella* (~3.07 – 7.08 log cfu/ml) except for both *S.* Anatum strains at pH 7.4 where numbers were reduced to below the limit of detection (>6.62 log cfu/ml). *Salmonella* numbers were reduced to be less than the limit of detection after heat shock in all meat juices at pH 7.4 and in beef juice at pH 5.9. By contrast *Salmonella* numbers after heat shock were reduced to less than the limit of detection (>7.22 log cfu/ml) for all treatments in PBS irrespective of its pH. Meat juice constituents and pH may play a role in protecting *Salmonella* against the effect of heat treatment.

365

ARDaP: Improved antimicrobial resistance detection and prediction from whole-genome sequence data

Derek S Sarovich[1], Danielle Madden[1], Mehmet Birikmen[1], Kim Schabacker[1], Erin P Price[1]

1. University of the Sunshine Coast, Sippy Downs, QLD, Australia

Whole-genome sequencing (WGS) is rapidly changing the clinical microbiology landscape and will eventually become a mainstream diagnostic tool. To this end, there has been a shift towards interpreting an isolate’s phenotypic profile based on the genomic sequence, effectively circumventing the need for multiple diagnostic assays or traditional culture-based methods that can be laborious, costly, inaccurate and have long turn-around-times. Numerous efforts have been undertaken to design software capable of predicting an antibiotic resistance profile based on sequence data; however, current algorithms often imprecisely predict resistance profiles for several reasons. Detection of small insertion-deletions (indels), copy number variants and gene loss have received surprising little attention given the importance of these genetic alterations in conferring antibiotic resistance. Additionally, all current methods can only detect known resistance mechanisms and are incapable of predicting if a novel genetic variant is likely to result in antibiotic resistance. We present an improved algorithm for Antibiotic Resistance Detection and Prediction from WGS data (ARDaP). The applicability of our approach was validated using *Pseudomonas aeruginosa* as a model organism due to the complexity of resistance mechanisms present in this species. We demonstrate that ARDaP can accurately identify the presence of any known resistance mechanism, identify novel genetic variants that could lead to increased risk of antibiotic resistance using a predictive, probability mapping approach and reports the predicted antibiotic resistance profile in an easy to interpret, clinically focused report.

366

Silver Nanoparticles: Green Synthesis and their Effective Applications

SHABANA SHABANA[1], Ambreen Ahmed[1]

1. Department of Botany, University of the Punjab, Lahore, Pakistan, Lahore, PUNJAB, Pakistan

Abstract

Nanotechnology is one of the fastest growing technologies with multidisciplinary uses. Green production of metal nanoparticles can revolutionize the world by giving an ecofriendly substitute to chemical and physical methods of nanoparticles synthesis that are quite toxic and expensive. Current study deals with the production of silver nanoparticles through auxin producing plant growth promoting rhizobacteria (PGPR). These biosynthesized silver nanoparticles are evaluated for their antibacterial activity and plant growth promotion potential. The strains *Bacillus cereus* (S12) and *Bacillus subtilis* (Mt3b) used in the current study showed excellent potential for silver nanoparticles biosynthesis which exhibit antibacterial potential. Maximum inhibition zone of 1mm was recorded with these silver nanoparticles. Nanoparticles synthesized were evaluated for their growth promotional potential and their application improved growth of *Triticum aestivum* L. with improvement in shoot length, root length, number of leaves and fresh weight significantly as

compared to control plants. Thus green production of nanoparticles is an ecofriendly and economic approach which can be used as antibacterial agent and nanobiofetilizers effectively.

367

Comparison of Copan WASP™ versus BD Kiestra™ InoqulA in Examination Time for Positive Urine Culture

Mir H Noorbakhsh[1], Susan Sharp[2], Susan Novak-Weekley[2], Jacob Nelson[2]

1. Sutter Health Shared Laboratory, Livermore, CA, United States

2. Copan Diagnostics, Inc., Murrieta, CA, USA

Background: Automated plating instruments are available to reduce labor associated with specimen inoculation and improve the quality of plating. The two most common instruments on the market are the WASP (Copan Diagnostics) and Kiestra InoqulA (Becton Dickinson). The impact of inoculum size differences (1 µL WASP, 10 µL for Kiestra) was studied on the time necessary for reading cultures.

Methods: 50 specimens submitted for routine culture were inoculated by WASP onto a Blood-Agar/MacConkey (BAP/MAC) bi-plate using a dual 1µL loop with streaking pattern WASP1, and on BAP and MAC whole plates using a 1µL loop with streaking pattern WASP2. They were also plated using the InoqulA onto BAP and MAC whole plates using 10 µL with streaking pattern KI1. All plates were incubated in the WASPLab™ (CO_2) incubator for 18 hours. Technologists were presented with images for WASP1, WASP2 and KI1 (total of 150 images for each). The examination time of each set of plates was recorded and averaged between three technologists.

Results: Average technologist read times for each inoculated set of plates was 17.47 minutes (WASP1), 19.04 minutes (WASP2), and 28.78 minutes (KI1). When compared to the KI1 set, the WASP1 set was read 11.31 minutes faster and the WASP2 set 9.74 minutes faster. Given an average technologist wage of $58.59/hr within the Sutter Health System (SHS) the potential labor cost savings is approximately $22.09/100 urine cultures for WASP1 and $19.02/100 urine cultures for WASP2, compared to KI1.

Conclusions: Technologists within SHS analyzed plates processed on WASP faster than those processed on InoqulA. We believe the improvement in reading times for plates inoculated with the WASP is reflective of better isolation of colonies along with easier calculation of colony counts due to the 1µl volume used for plating. SHS processes 700 urines/day and using either the WASP1 or WASP2 streaking patterns was shown to provide an approximate labor savings (excluding benefits) of $56,439.95/year and $48,596.10/year, respectively, compared to KI1.

1. 1. Bourbeau, PP, N. Ledeboer. 2013. Automation in Clinical Microbiology. J. Clin. Microbiol. 51:1658-1665.

2. 2. Quiblier C, Jetter M, Rominski M, Mouttet F, Bottger EC, Keller PM, Hombach M. 2016. Performance of Copan WASP for Routine Urine Microbiology. J. Clin. Microbiol. 54:585-592.

368

Engineering a bacterial toxin for improved function as a *N*-glycolylneuraminic acid specific lectin

Lucy K Shewell[1], Jing Wang[1], Adrienne W Paton[2], James C Paton[2], Christopher J Day[1], Michael Jennings[1]

1. Institute for Glycomics, Griffith University, GOLD COAST, QLD, Australia

2. Research Centre for Infectious Diseases, University of Adelaide, Adelaide, SA, Australia

The B subunit of the subtilase cytotoxin (SubB) produced by Shiga toxigenic *Escherichia coli* (STEC) recognises *N*-glycolylneuraminic acid (Neu5Gc) containing glycans, the most prominent form of aberrant glycosylation in human cancers. We have previously engineered SubB by construction of a $SubB_{\Delta S106/\Delta T107}$ mutant (SubB2M) for greater specificity and enhanced recognition of Neu5Gc containing glycans. In this study, we further explore the utility of SubB2M as a Neu5Gc lectin by showing its improved specificity and recognition for Neu5Gc containing glycans over the wild-type SubB protein and an anti-Neu5Gc IgY antibody in a *N*-acetylneuraminic acid (Neu5Ac)/Neu5Gc glycan array and by surface plasmon resonance. Far-western blot analysis showed that SubB2M preferentially binds to bovine serum glycoproteins over human serum glycoproteins. SubB2M was also able to detect Neu5Gc containing bovine glycoproteins spiked into normal human serum with greater sensitivity than the wild-type SubB and the anti-Neu5Gc IgY antibody. These results suggest that SubB2M will be a useful tool for the testing of serum and other bodily fluids for cancer diagnosis and prognosis.

369

Uropathogen Detection Comparison of Enhanced Quantitative Urine Culture Protocol and Standard Urine Culture Protocol in Patients with Urinary Tract Infection: Evidence Based Case Report

Samuel Dominggus Chandra Siahaan[1], Ginova Nainggolan[2]

1. Faculty of Medicine, Universitas Indonesia, Jakarta Pusat, DKI Jakarta, Indonesia

2. Renal and Hypertension Division of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta Pusat, DKI Jakarta, Indonesia

Background: Good management of urinary tract infection is strongly supported by the detection of uropathogen in patients' urine. Untill now, the standard protocol of urine culture is known as the gold standard for uropathogen detection. Currently, a new protocol known as the Enhanced Quantitative Urine Culture (EQUC) protocol is considered capable of optimizing uropathogen detection in patients with urinary tract infections.

Objective: To find out whether the EQUC protocol can optimize uropathogen detection compared to the standard urine culture protocol.

Method: Literature search was done with the help of boolean operator in several medical journals such as Pubmed, Proquest, Cochrane, and Clinicalkey. Literature found from the search was appraised using validity, importance, and applicability (VIA) method according to the Oxford Centre of Evidence-Based Medicine critical appraisal worksheet.

Result: The literature search had found one appropriate literature suitable for the critical appraisal. After critical analysis, EQUC protocol has a sensitivity of 63.7%, specificity of 84.1%, positive predictive value (PPV) of 90.9%, and negative predictive value (NPV) of 48.2% compared to the standard urine culture protocol. The EQUC protocol is also capable of detecting 110 variations of uropathogen from 75 urine samples, while standard protocol is only capable of detecting 55 (50%) variations of uropathogen.

Conclusions: EQUC is considered to be better in term of variability for uropathogen detection compared to the standard urine culture protocol and good enough to replace standard urine culture protocol in term of diagnostic value, but is considered not to replace the standard urine culture protocol in terms of cost and time required.

1. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of action and treatment options. Nat Rev Microbiol. 2015 May; 13(5):269-84.

2. Kasper DL, Fauci AS, Hauser SL, Longo DL, Jameson JL, Loscalzo J. Harrison's principles of internal medicine. 19th ed. New York:McGraw-Hill; 2015.

3. Colgan R, Nicolle LE, McGlone A, Hooton TM. Asymptomatic bacteria in adults. Am Fam Physician. 2016 Sep; 74(6):985-90.

4. Price TK, et al. The clinical urine culture: enhanced techniques improve detection of clinically relevant microorganisms. J Clin Microbiol. 2016 May; 54(5):1216-22.

370

Potential pathogens identified in lung samples from pigs with pleurisy at an abattoir in Queensland Australia

Conny Turni, Tamsin Barnes, Joanne Meers, Kit Parke, Reema Singh, Sarah Yee, Jillian Templeton, Kyaw Mone, Peter McKenzie, Pat Blackall

Pleurisy has a huge impact in the Australian pig industry at both the production stage and the abattoir. In recent years, an increase in pleurisy at the abattoir has been noted in Australia and overseas. Yet there is limited knowledge of the bacteria and viruses involved in pleurisy in Australia. Hence this study investigated the bacterial agents and porcine circovirus-2 found in lungs and lymph nodes, respectively, in slaughter pigs with pleurisy from a South-East Queensland abattoir. Forty-six farms were sampled with five lungs from pigs with pleurisy sampled from each farm. The lungs were scored, and the presence of key pathogens in the lungs determined. Lungs were sampled, cultured onto BA/SN plates and the growth being scored. The most prevalent bacteria were *Mycoplasma hyopneumoniae* and *Streptococcus suis*, found from 34 and 38 farms, respectively. Thirty-one farms had positive results for PCV2. Other bacteria were *Actinobacillus* species (29 farms), *Pasteurella multocida* (24 farms), *Mycoplasma flocculare* (9 farms), *Actinobacillus pleuropneumoniae* (7 farms), *Mycoplasma hyorhinis* (4 farms), *Haemophilus parasuis* (1 farm), Bisgaaard Taxon 10 (1 farm), *Streptococcus minor* (1 farm) and *Streptococcus porcinus* (1 farm). Most of the farms had more than one species of bacteria detected. Most of the bacteria were in low numbers, however, some species such as *S. suis*, *P. multocida* and *A. pleuropneumoniae*, were in high numbers. These species are associated with chronic respiratory disease and are common in pigs with pleurisy at the abattoirs in other countries.

371

Investigating the risk of human disease from parasites of small mammals and bats in Cambodia

Ian H Mendenhall[1], Sophie Borthwick[1], Alan Hitch[2], Dany Chheang[3], Gavin JD Smith[1]

1. Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore

2. Museum of Wildlife and Fish Biology, University of California, Davis, CA, USA

3. Department of Wildlife and Biodiversity Conservation, Forestry Administration, Phnom Penh, Cambodia

Emerging and re-emerging infectious diseases pose a significant public health challenge, with severe economic, social, and health consequences. The frequency of epidemics caused by newly emerging and re-emerging pathogens and the likelihood of rapid global spread have increased dramatically in recent decades, with Southeast Asia considered a hot spot for future emergence events. Small mammals and bats play an important role in the maintenance and transmission of several zoonotic agents, such as filoviruses, coronaviruses, and henipaviruses. Cambodia is one of 35 global biodiversity hotspots that provides habitats for thousands of wildlife species, including over 70 species of bats. By proactively sampling animal populations in Cambodia to discern circulating parasitic genotypes and screening human sera for evidence of exposure, we aim to determine those parasites with human pathogenic potential. A standardised trapping regimen has been applied to allow us to understand ecological and environmental variables associated with host and parasite presence-absence, facilitating the creation of ecological niche maps and risk models to inform future surveillance efforts across Southeast Asia.

372

High susceptibility and vector competence of Australian *Aedes aegypti* to Zika virus

Liesel Stassen[1], Leon E Hugo[2], O'mezie Ekwudu[2, 1], Elvina Viennet [3], Helen M Faddy [3], Gregor J Devine[2], Francesca D Frentiu[1]

1. Queensland University of Technology, Brisbane, QLD, Australia

2. Mosquito Control Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

3. Australian Red Cross Blood Service, Brisbane, QLD, Australia

Recent epidemics of Zika virus (ZIKV) in the Pacific and the Americas have highlighted its potential as an emerging pathogen of global importance. ZIKV is a member of the family *Flaviviridae*, genus *Flavivirus*, and is transmitted to humans by *Aedes* mosquito species. Both *Ae. aegypti* and *Ae. albopictus* are known to transmit ZIKV. However, variable vector competence has been observed between mosquito populations from different geographical regions. Australia remains at risk of ZIKV introduction due to its close proximity to the Western Pacific, the presence of susceptible mosquito vectors in Northern

Queensland, and favorable environmental conditions. Therefore, we evaluated the vector competence of *Ae. aegypti* from Queensland and *Ae. albopictus* from the Torres Strait Islands, for a Brazilian epidemic strain of ZIKV. Mosquitoes were exposed to an infectious blood meal, and reared at either constant or fluctuating temperatures (mean of 28°C). Virus RNA copies in mosquito bodies, distal tissues (wings and legs) and saliva were quantified post infection. Overall, both species supported high viral body titers (>10^7 copies/mosquito body) at 14 days post infection (dpi), with no significant differences in viral copy number between constant and cyclic temperature regimes. Infection rates at 14 dpi were similar for both species. Despite high infection rates in both vectors, the transmission rates of ZIKV to saliva in *Ae. aegypti* (60%) was significantly higher than in *Ae. albopictus* (10%) at 14 dpi. A significant difference in viral copy number in wings and legs between species was observed, with higher titers in *Ae. aegypti*. Exposure to constant versus fluctuating temperature also had a significant effect on viral titer in *Ae. aegypti*. Our results are in agreement with the role of *Ae. aegypti* in the global emergence of ZIKV, and suggest that *Ae. aegypti* are more competent for transmission of ZIKV than *Ae. albopictus*. Therefore, *Ae. aegypti* is likely to be the primary potential vector of ZIKV in Australia, which should be taken into account during disease risk evaluation.

373

Seeding of the fetal gut microbiome: insights into origins and significance

Lisa Stinson[1], Jeff Keelan[1], Matthew Payne[1]

1. Faculty of Medicine, Division of Obstetrics and Gynaecology, University of Western Australia, Perth, WA, Australia

Background: Early-life microbial colonisation is believed to play a role in immune programming and later life health, yet the evidence regarding the origins, timing and significance of the neonatal microbiome remains inconclusive due to problematic study design, biased amplification and contamination.

Method: Placental, amniotic fluid, first-pass meconium and cord blood samples were collected from 50 elective Caesarean section deliveries. An optimised sampling and analysis protocol was employed to minimise/control for contamination, allowing characterisation of the fetal gut microbiome and its relationship with maternal parameters, short chain fatty acid (SCFA) levels and inflammation.

Results: All meconium samples contained detectable levels of bacterial DNA and the immunomodulatory SCFAs acetate and propionate, confirming the hypothesis that the fetal gut is inoculated with bacteria/bacterial DNA *in utero*. At the phylum level, meconium was dominated by Proteobacteria and Firmicutes. Acinetobacter - known to be allergy protective - was the most abundant genus (found in 95% of samples). Importantly, this genus has been found in the core non-pregnant endometrial microbiome, but not paired vaginal samples. Lactobacillus (which dominates the vaginal microbiome) was found in only 5 samples. Presence and abundance of Acinetobacter DNA was associated with elevated immune responses to 5'ppp-dsDNA (a RIG-I agonist). Maternal diabetes, intra-amniotic inflammation and SCFA levels were all associated with altered meconium microbiome.

Conclusions: Seeding of the fetal gut microbiome commences prenatally and may originate from the endometrial microbiome present at time of conception; vaginal contribution appears minimal. Maternal metabolic health and intrauterine inflammation may influence fetal immune programming via modulation of the fetal microbiome and immunomodulatory SCFAs.

374

Molecular characterisation of PPVP, a key effector molecule within the LirA regulon of *Mycobacterium tuberculosis*

Miljan Stupar[1], Jane Anderson[1], Nick West[1]

1. School of Chemistry and Molecular Biosciences; and Infectious Disease Research Centre, University of Queensland , Brisbane , QLD, Australia

Given the enormous health burden of Tuberculosis, great effort has been invested into understanding the molecular mechanisms underpinning mycobacterial pathogenesis. Approximately 10% of the coding capacity of the *Mycobacterium tuberculosis* genome is dedicated to the PE/PPE family of proteins, a conserved set of functionally distinct proteins that contain a characteristic Pro-Glu (PE) or Pro-Pro-Glu (PPE) motif in their N-terminal region. One member, referred to here as PPVP, appears to be under the control of LirAB, a newly characterised two-component regulatory system of *M. tuberculosis*. RT-qPCR data demonstrates that *ppvp* is downregulated under acidic conditions *in vitro*, suggesting this protein may be important for adaptation to the macrophage phagosome. Overexpression of *ppvp* in recombinant *Mycobacterium smegmatis*, an avirulent relative of *M. tuberculosis*, is associated with increased survival, enhanced phagocytosis and immunomodulation in murine macrophages. Molecular tracking of PPVP using confocal microscopy indicates this protein localises to the nuclear membrane following internalisation of murine macrophages. Our work suggests a multi-functional role of PPVP which may mediate adherence and evasion of host-mediated immune factors.

375

Evolution of influenza A/H3N2 virus in Singapore

Yvonne Su[1], Ramandeep Virk[1], Hong Kai Lee[2], Ian Mendenhall[1], Pauline Lam[1], Jessica Kok[1], Gavin Smith[1]

1. Duke-NUS Medical School, Singapore, SINGAPORE

2. Molecular Diagnostic Centre, National University Hospital, Singapore

Influenza seasonality in Singapore shows biannual peaks that coincide with the winters of temperate countries. Human A/H3N2 virus has been circulating in humans for five decades and the virus continues to cause seasonal epidemics worldwide. Antigenic drift is an underlying evolutionary mechanism that accumulates mutations in the viral genomes, allowing the viruses to escape host immune response. In order to understand the current evolution of influenza virus in Singapore, we collected a total of 313 human influenza samples that from National University Hospital (NUH) during March 2016–July 2017. Out of these, 82 samples were identified as A/H1N1, 186 as A/H3N2, 44 as influenza B viruses, with one un-determined. Positive A/H3N2 samples were selected for NGS sequencing. Viral RNA was isolated and genome-wide RT-PCR amplification of influenza A viruses was carried out using primers targeting conserved 5' and 3' regions. We obtained sufficient NGS coverage for all eight segments of influenza A viruses, and 62 novel A/H3N2 genomes were generated. Gene phylogenies were reconstructed using maximum likelihood method in RAxML v8.0. For the 2016–17 season, A/H3N2 virus was predominant in Singapore, and co-circulated with A/H1N1 and influenza B viruses. Phylogenetic analysis of the HA gene shows that our recent A/H3N2 isolates fall into two clades 3C.2a and 3C.2a1, indicating the Singapore viruses are inter-mingled with global sequences. A/H3N2 virus has drifted significantly in the 2016–17 season, that has led to an update of the WHO influenza virus strain (A/Singapore/INFIMH-16-0019/2016-like) for the 2018 Southern Hemisphere.

Poster Abstracts

376

Heparan sulfate mimetic compounds in modulating RRV as potential therapies

Aroon Supramaniam[1], Vito Ferro[2, 3], Lara Herrero[1]

1. Griffith University, Parkwood, QLD, Australia

2. School of Chemistry & Molecular Biosciences, University of Queensland, Brisbane, Queensland, Australia

3. Australian Infectious Diseases Research Centre, University of Queensland, Brisbane, Queensland, Australia

Introduction: Arthritogenic Alphavirus, such as Ross River virus (RRV) and Barmah Forest virus (BFV), are transmitted by mosquito vectors and cause musculoskeletal manifestations. Patients experience excruciating pain and inflammation of their joints and surrounding muscle tissues (1). Current treatments for arthritogenic alphaviruses only provide temporary or partial relief. Methods: Herein, we describe the use of PG545 or the treatment of RRV induced arthritogenic disease. We evaluated the treatment efficacy of this representative compound in a mouse model of RRVD. We evaluated their joint dysfunction, expression levels of both host soluble factors and components of the cartilage matrix, viral titer and histopathology in viral specific target organs. Results: Prophylactic compound treated RRV infected mice had significantly reduced viral loads in target organs corresponding to a reduction in their clinical scores of limb weakness and immune infiltrate recruitment. At peak disease, compound treated RRV mice had lower expression levels of host factors IL-6 and CCL-2. In addition, treatment also demonstrated protection in muscle fibres and hyaline cartilage structure. Conclusion: Taken together these findings suggest that the HS mimetic compound may have a direct inhibitory effect on both RRV infection as well as the RRV-induced inflammatory disease in host organisms. This suggests a dual mode of action in its efficacy to treat RRV infection and disease indicating a potential to treat patients who suffer both acute and chronic symptoms.

1. Supramaniam A, Liu X, Ferro V, Herrero LJ. 2018. Prophylactic antiheparanase activity by PG545 is antiviral in vitro and protects against Ross River virus disease in mice. Antimicrob Agents Chemother 62:e01959-17. https://doi.org/10.1128/AAC.01959-17.

377

Faecal Strongyloides stercoralis real-time polymerase chain reaction assay validation study

Christopher D Swan[1], Genevieve L McKew[2], Thuy Phan[2]

1. Infectious Diseases and Microbiology, Royal North Shore Hospital, St. Leonards, New South Wales, Sydney

2. Infectious Diseases and Microbiology, Concord Repatriation General Hospital, Sydney, New South Wales, Australia

Strongyloides stercoralis is a nematode endemic to subtropical and tropical regions which may cause asymptomatic carriage, peripheral eosinophilia, cutaneous, gastrointestinal, and pulmonary disease, or hyperinfection syndrome. Conventional diagnostic methods for strongyloidiasis include faeces culture and microscopy, with low sensitivity in chronic infection due to the low helminth burden, and serology, which may be prone to false-negative results with immunocompromise and false-positive results with other infections and immunological disorders[1 2]. We evaluated a laboratory-developed real-time polymerase chain reaction (qPCR), detecting the 18S ribosomal RNA gene, compared to conventional diagnostic methods, using serology via enzyme-linked immunosorbent assay (ELISA) as the gold-standard. The population studied was tertiary hospital inpatients and outpatients residing in a non-endemic area. 750 unfixed stool specimens submitted between 2014 and 2018 were tested for *S. stercoralis* via microscopy and qPCR. Agar plate culture (APC), Harada-Mori culture (HMC), and ELISA were performed in conjunction with 141, 135, and 177 of the specimens respectively. qPCR yielded 13 positive and 730 negative results whilst inhibition occurred in 7 specimens. ELISA yielded 53 positive, 18 equivocal, and 106 negative results. Results for direct diagnostic methods obtained following treatment with ivermectin were excluded from the performance analysis. Compared with ELISA, qPCR, microscopy, APC, and HMC exhibited sensitivities of 38% (13/34), 6% (2/33), 3% (1/29), and 0% (0/24) respectively and specificities of 100%. Our results conflict a meta-analysis in which molecular methods exhibited a sensitivity and specificity of 56.50% and 95.38% respectively compared with parasitological and serological methods, although only 2 of the 14 included studies were performed in non-endemic areas[3]. There is a paucity of data from non-endemic settings but our results are comparable to those of one study performed in a non-endemic area where APC, qPCR, and serology exhibited sensitivities of 45%, 57%, and 95% respectively[4], most likely reflecting the low helminth burden in chronic strongyloidiasis. We recommend employing a combination of molecular, parasitological, and serological methods for diagnosis and screening of strongyloidiasis.

1. van Doorn HR, Koelewijn R, Hofwegen H, et al. Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans. J Clin Microbiol 2007;45(2):438-42. doi: 10.1128/JCM.01735-06

2. Bisoffi Z, Buonfrate D, Sequi M, et al. Diagnostic accuracy of five serologic tests for Strongyloides stercoralis infection. PLoS Negl Trop Dis 2014;8(1):e2640. doi: 10.1371/journal.pntd.0002640

3. Buonfrate D, Requena-Mendez A, Angheben A, et al. Accuracy of molecular biology techniques for the diagnosis of Strongyloides stercoralis infection-A systematic review and meta-analysis. PLoS Negl Trop Dis 2018;12(2):e0006229. doi: 10.1371/journal.pntd.0006229

4. Buonfrate D, Perandin F, Formenti F, et al. A retrospective study comparing agar plate culture, indirect immunofluorescence and real-time PCR for the diagnosis of Strongyloides stercoralis infection. Parasitology 2017;144(6):812-16. doi: 10.1017/S0031182016002559

378

Nuc is an extracellular nuclease that mediates competence and biofilm formation in *Moraxella catarrhalis*

Aimee Tan[1], Luke V Blakeway[1], Wing Li[1], Kate L Seib[1]

1. Griffith University, Gold Coast, QLD, Australia

Moraxella catarrhalis is a Gram-negative bacterial pathogen, and one of the major bacterial causes of otitis media and exacerbations of chronic obstructive pulmonary disease along with *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae*. However, unlike the latter pathogens, no vaccine yet exists for *M. catarrhalis*, and many aspects of its physiology and pathogenesis remain unexplored. One example of this is that *M. catarrhalis* exhibits potent extracellular nuclease activity, which can be used to discriminate it from other Gram-negative cocci, but the genetic basis of this activity has not been investigated. In contrast, extracellular nucleases have been studied in both *S. pneumoniae* (EndA) and *H. influenzae* (Nuc), where they mediate virulence mechanisms such as biofilm formation and resistance to neutrophil killing, via destruction of extracellular traps.

Our work investigated a putative open reading frame in *M. catarrhalis* and showed that it encoded an extracellular factor that digests both DNA and RNA, that we have subsequently termed Nuc. Nuc also facilitates the natural competence of *M. catarrhalis*, with *Δnuc* mutant strains severely attenuated in transformation efficacy assays. Whilst *nuc* is not essential to the growth of the cell, *Δnuc* mutant strains show increased aggregation compared to parental strains. Similarly, *Δnuc* mutant strains accumulate increased biomass in static biofilm assays, suggesting the nuclease is essential for the correct formation and dispersal of *M. catarrhalis* biofilms. These phenotypes suggest that Nuc is important for the virulence of *M. catarrhalis* in infections, as has been seen with nucleases in other otopathogens *S. pneumoniae* and *H. influenzae*.

379

Adaptation Strategies Inferred From Resource Allocation in Cyanobacterial Membrane Proteomes

Fallen Teoh[1], Martin Ostrowski[1], Ian Paulsen[1]

1. Molecular Sciences, Macquarie University, Sydney, NSW, Australia

Marine cyanobacteria, such as *Synechococcus* are among the most abundant and widespread primary producers in the open ocean. *Synechococcus* strains belonging to different clades have adapted distinct strategies for survival across a range of marine niches: from turbid, estuarine waters to transparent oligotrophic waters and various temperatures across 120^0 of latitude. Clades I and IV are prevalent in colder, mesotrophic, coastal waters, while clades II and III show preference for the warmer, oligotrophic open oceans. To gain insight into the relative resources these unicellular organisms invest into adaptation strategies we performed shotgun proteomics, focusing on the membrane proteomes, of four *Synechococcus spp.* strains namely CC9311 (clade I), CC9605 (clade II), WH8102 (clade III) and CC9902 (clade IV). More than 600 distinct proteins were identified in membrane extracts of each species and their relative expression levels were determined using label-free quantification. Membrane transporter systems were abundant in all strains, with 63-92 membrane transport proteins identified and accounted for 8-17% of overall proteins expression. Comparative membrane proteome analysis showed that CC9902 and WH8102 have a significant resource investment in phosphate uptake, which represent 44% and 38% of its own overall transporter protein expression. WH8102 displayed high expression of the FutA (iron ABC transporter substrate binding protein) suggested that high binding affinity of iron is a key adaptation strategy in oligotrophic environments. One protein annotated as a phosphatase 2C enzyme was very highly expressed in the temperate/coastal strains CC9311 and CC9902, suggested this protein serves a vital, but yet undefined function, for strains living in these environments. Overall, this study suggested that membrane transporters and sensing systems are key components in the different adaptation strategies of cyanobacteria.

380

Comparison of the highly multiplexed PlexPCR® RespiVirus 11 (beta) assay with a commercial assay

Priya Thaivalappil[1], Sandhya Nair M Rajan[1], Nicole Lima[1], Litty Tan[1], Elisa Mokany[1], Meik Dilcher[2], Kevin Barratt[2]

1. SpeeDx, Sydney, NSW, Australia

2. Virology/Serology Department, Canterbury Health Laboratories, Christchurch, New Zealand

Background: Economic and seasonally-driven pressures for faster, accurate sample testing at reduced costs have resulted in an increased need for highly sensitive and specific assays with high throughput for respiratory illnesses. The PlexPCR® RespiVirus 11 (beta) assay (SpeeDx, Australia) detects 11 respiratory viral targets in a two-well format, using PlexZymes to enable highly sensitive and efficient qPCR multiplexing (1). Here we evaluated this assay in comparison with the Fast Track Diagnostics Respiratory pathogens 21 (FTD RP21) multiplex qPCR assay.

Material/Methods: PlexPCR® RespiVirus 11 (beta) assay was evaluated against FTD RP21 assay on 245 retrospective upper and lower respiratory tract samples. Targets common to both assays, Influenza A (FluA), Influenza B (FluB), Rhinoviruses (RhV), Respiratory Syncytial Viruses A/B (RSV), Human metapneumovirus (hMPV), Adenoviruses B/C (AdV (B/C)) and Human parainfluenza viruses 1, 2, 3 4 (HPIV 1-4) were analysed. Discrepant samples were resolved by re-testing with the FTD RP21 assay.

Results: Compared to FTD RP21 assay, the PlexPCR® RespiVirus 11 (beta) assay had a final sensitivity/specificity of: FluA 92%/99.41%, FluB 100%/99.51%, RSV 100%/100%, RhV 100%/99.12%, hMPV 90%/100%, AdV (B/C) 100%/99.15% and HPIV 1-4 100%/100%. Processing of the 245 samples required 502 wells (5.3 plates) for PlexPCR® RespiVirus 11 (beta) assay versus 1024 wells (10.6 plates) for FTD RP21 assay.

Conclusions: The PlexPCR® RespiVirus 11 (beta) assay demonstrated excellent sensitivity and specificity for the detection of 11 respiratory viral targets. In addition the assay is highly multiplexed and could be run in 2 wells versus 4 wells as for the comparator commercial assay, allowing significant gains in increasing throughput and reducing costs.

1. (1) Mokany et al (2013) Clin. Chem. 59:419

Poster Abstracts

381

Molecular characterization of fluoroquinolone-resistant avian pathogenic *Escherichia coli* isolated from diseased chickens in Thailand

Thotsapol Thomrongsuwannakij[1], Niwat Chansiripornchai[1]

1. Department of Veterinary Medicine, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of colbacillosis in poultry causing economical losses in this industry worldwide. Eighty-four isolates of APEC isolated from diseased chickens in Thailand were examined for their antimicrobial susceptibility. Thirty-four isolates, which were resistance to nalidixic acid or enrofloxacin, were characterized for their mechanisms of fluoroquinolone resistance. In total, isolates demonstrated resistance to tetracycline (84.52%), amoxicillin (70.24%), doxycycline (64.29%), trimethoprim-sulfamethoxazole (51.19%), nalidixic acid (40.48%), enrofloxacin (30.95%), chloramphenicol (28.57%), and gentamicin (9.52%). Twenty-nine isolates contained a mutation in *gyrA* of a serine at position 83 to a leucine (Ser-83-Leu), and 23 isolates contained a mutation of Asp87 to one one of three amino acids: Asn (n=18), Tyr (n=4) or Gly (n=1). No mutation was found in *gyrB*. Eighteen isolates contained a mutation in *parC* of a Ser80 to an isoleucine, and an isolate contained a mutation of Ser87 to an arginine. Furthermore, other point mutations were also found in *parC* such as Ala-56-Thr (n=1), and Glu-84-Gly (n=1). Six isolates contained a mutation in *parE* including Ser-458-Ala (n=4), Asp-475-Glu (n=1), and Ile-464-Phe (n=1). In conclusion, the high-level fluoroquinolone resistance in APEC has a correlation with high numbers of mutations in quinolone resistance-determining region (QRDR).

382

Overdiagnosis of rotavirus infection due to vaccine virus shedding.

Sarah Tozer[1], David Whiley[2, 3], Sophia Ye[4, 3], Julia Clark[5], Cheryl Bletchly[3], Stephen Lambert[6], Keith Grimwood[7], Graeme Nimmo[3]

1. QPID - Childrens Health Queensland, Childrens Health Queensland, Brisbane, QLD, Australia

2. Child Heath Research Center, University of Queensland , South Brisbane, Qld, Australia

3. Pathology Queensland, Queensland Health, Brisbane, Queensland, Australia

4. UQCCR - Herston, University of Queensland , Brisbane, Qld, Australia

5. Infection Management and Prevention Services, Lady Cilento Children's Hospital, South Brisbane, Queensland, Australia

6. Communicable Diseases Branch, Queensland Health, Brisbane, Queensland, Australia

7. Infectious Diseases, Griffith University and Gold Coast Health, Gold Coast, Queensland, Australia

Introduction: In a previous community-based birth cohort study, we observed that shedding of the Rotavirus RV5 vaccine virus was frequent and prolonged (several weeks duration) in the stool samples of vaccinated infants. Prompted by recent increases in rotavirus identification in Queensland, we investigated whether detection of vaccine shedding may impact upon routine rotavirus diagnosis and surveillance.

Methods: Available stool samples (n = 79) testing positive by rotavirus PCR at Pathology Queensland in the period late 2016 to mid 2017 were subject to rotavirus genotyping to distinguish RV5 vaccine virus from wildtype virus. The patients comprised 37 females and 42 males, ranged in age from 2 weeks to 89 years (average 34 years); 21 were less than 1 year old, by which age all three RV5 vaccine doses are administered.

Results:

Genotyping showed that of the 79 rotavirus-PCR positives samples, 6 were RV5, 1 comprised a mix of RV5 and wildtype virus and 46 were wildtype virus. A further 26 could not be genotyped, primarily because the viral load was too low. All seven RV5 detections were from children less than 1 year old; for this age group RV5 comprised 33% (7/21) of all detections.

Conclusion:

These preliminary data suggest that detection of vaccine virus may lead to overdiagnosis of rotavirus infection in infants, and likely accounts for some (but not all) of the recent increases in numbers. The data highlight the need for screening methods to distinguish vaccine from wildtype virus. Studies are ongoing.

Disclosure of Interest Statement:
D Whiley receives research funding from SpeeDx Pty Ltd. No pharmaceutical grants were received for the development of this study.

383

Are peri-urban wild dogs another potential source of transmission of Q fever in Queensland?

Sarah J Tozer[1], Caitlin Wood[1, 2], Lana Harriott[2], Ricardo Soares[2, 3], Rowland Cobbold[2], Nigel Perkins[2]

1. QPID - Childrens Health Queensland, Childrens Health Queensland, Brisbane, QLD, Australia

2. School of Veterinary Science, University of Queensland , Gatton, Qld, Australia

3. Child Heath Research Center, University of Queensland , South Brisbane, Qld, Australia

Q fever disease caused by infection with *Coxiella burnetii,* is endemic in Southeast Queensland, Australia. *C. burnetii* has been identified in a wide range of animals including traditional sources such as cattle, sheep and goats but also, domestic mammals, Australian marsupials and ticks. Limited data exists on wild dog populations in Queensland and the role that these animals may play in *C.burnetii* transmission to livestock, and potentially to humans.

This study aims to investigate evidence of *C. burnetii* exposure and bacterial shedding in a convenience set of samples collected from wild dogs roaming Southeast Queensland, Australia. Samples were obtained from peri-urban wild dogs captured as part of a large pest management program conducted in Southeast Queensland between August 2012 and May 2015. Sera from 39 canines was investigated using IFA for *C.burnetii* exposure and direct transmission was assessed by screening 107 faecal samples using PCR with dual targets specific to *C. burnetii, IS1111 and Com1*. Whole blood from 77

canines were also screened using real-time PCR for dual targets. Geographical locations of wild dogs included in our sample sets were mapped. Preliminary serological screening detected evidence of *C. burnetii* exposure in dogs across a wide geographical area while faecal samples showed evidence of bacterial dissemination into the environment.

The results of this study provide insights into the potential role of peri-urban, wild dogs as a reservoir and possible source of *C. burnetii* transmission in Queensland.

384

Study for Monitoring Antimicrobial Resistance Trends (SMART) in Australia and New Zealand (ANZ), 2016-2017

Robert Badal[1], Geoff Coombs [2], Dragana Drinkovic[3], Justin Ellem [4], Narelle George [5], Andree Hubber [6], Tony Korman[7], Sally Roberts [8], Susan Taylor [9], Merrin Tulloch[6]

1. IHMA Inc , Schaumberg, IL, USA
2. Murdoch University , Perth , WA , Australia
3. North Shore Hospital , Auckland , New Zealand
4. Westmead Hospital, Sydney , NSW, Australia
5. Pathology Queensland , Brisbane , QLD, Australia
6. MSD, Macquarie Park, NSW, Australia
7. Monash Health , Melbourne, VIC , Australia
8. Auckland City Hospital , Auckland , New Zealand
9. Middlemore Hospital , Auckland, New Zealand

SMART has monitored the *in vitro* susceptibility patterns of clinical Gram-negative bacilli to antimicrobial agents in ANZ from intra-abdominal infections since 2002, urinary tract infections since 2009 and respiratory infections since 2015. This update includes suceptibility data from 2016-2017.

The top three pathogens for intra-abdominal infections (n=7112) were *Escherichia coli* (48%), *Klebsiella pneumoniae* (12%) and *Pseudomonas aeruginosa* (11%). Of these the most common sources were peritoneal fluid (36%), abscess (16%) and gallbladder (12%). The top three pathogens for patients with urinary infections (n=3530) were *E. coli* (57%), *K. pneumoniae* (14%) and *P. aeruginosa*(8%), and the most common sources were urine (93%) and kidney (6%). The top three pathogens from patients with respiratory infections (n=2085) were *P. aeruginosa* (35%), *E. coli* (14%), and *K. pneumoniae* (13%). Of these the most common sources were sputum (63%), endotracheal aspirate (27%) and bronchoalveolar lavage (9%). Excluding sputum isolates from respiratory samples the prevalence was *P. aeruginosa* (23%), *K. pneumoniae* (16%) and *E. coli* (15%).

Amongst 2833 Enterobacteriaceae from 2016-2017, rank order non-susceptibility (NS) was ceftriaxone (CRO, 20%), ceftazidime (CAZ, 18%), ciprofloxacin (CIP, 15%), piperacillin/tazobactam (PTZ, 13%), cefepime (FEP, 13%) and ceftolozane/tazobactam (C/T, 8%)†. NS to imipenem (IPM), meropenem (MEM) and amikacin (AMK) was ~1%. Of 212 CRO NS *E. coli* isolates from 2016-2017, rank order NS was PTZ (25%), C/T (17%), ETP (4%), AMK (7%), colistin (COL, 1%) and MEM or IPM (0%). Molecular analysis of *E. coli* (n=75) and *K. pneumoniae* (n=34) from 2016 confirms predominance of CTX-M-type extended-spectrum β-lactamases.

Amongst 727 *Pseudomonas aeruginosa* from 2016-2017, rank order NS was CIP (18%), PTZ (15%), FEP (13%), CAZ (13%), MEM (11%) and IPM (10%). NS to C/T and colistin was ≤ 3%. Of 81 MEM NS isolates, rank order NS was IPM (79%), TZP (60%), FEP (54%), CIP (49%), CAZ (48%), AMK (19%), C/T (17%) and COL (1%). Of 93 CAZ NS isolates, rank order NS was TZP (89%), FEP (69%), MEM (42%), CIP (39%), IPM (38%), C/T (22%), AMK (15%) and COL (1%). Molecular analysis of *P. aeruginosa* (n=67) isolates from 2016 identified a single acquired carbapenemase.

† EUCAST criteria

385

Glaesserella australis sp. nov., isolated from the lungs of pigs

Conny Turni[1], Nhan Giang[1], Vincent Wu[1], Lida Omaleki[1], Pat Blackall[1], Henrik Christensen

1. University of Queensland, Dutton Park, QLD, Australia

In recent years we received an increase of submission of haemophilic bacteria sourced from the respiratory tract of pigs to our reference laboratory for confirmation of identification. Due to the lack of discriminatory power of the routine 16S rDNA sequencing for closely related species within the *Pasteurellaceae* family, we analysed the 36 field isolates by using multi-locus sequence analysis (MLSA) of three housekeeping genes; *recN, rpoA* and *thdF*.

Geneious version 8.0.5 was used for aligning and similarity index calculation of the amplified genes to those from the type strains within the genera *Actinobacillus and Glaesserella*.

Among these 36 isolates a new species, consisting of 17 isolates, was identified with a genome similarity index of 0.56 to the closest related type strains – *Glaesserella parasuis* and *A. indolicus*. The type strains of *Glaesserella parasuis* and *A. indolicus* formed a group with nine isolates. A further seven isolates did not fit into a group due to lack of congruence of the *thdF* gene phylogeny with *recN*and *rpoA* and their identity remains uncertain.

Poster Abstracts

The 17 isolates of the potentially new species were further analysed using whole genome phylogeny. The candidate type strain (HS4635^T) of the potential new species shared 30.9% DNA-DNA homology with *Glaesserella parasuis*. The most outstanding biochemical difference between this new species with other members of the genera *Actinobacillus* and *Glaesserella* is the capacity to produce cytochrome C oxidase and indole, while lacking the capacity to produce catalase or urease. Like *Glaesserella parasuis* and some members of the genus *Actinobacillus*, this newly identified Gram-negative bacteria is satellitic on blood agar. Other key properties are acid formation from (-)-D-arabinose, (+)-D-galactose, (+)-D-maltose and trehalose, a failure to produce acid from (-)-D-mannitol and the production of b-galactosidase but not a-fucosidase. This new species is in the process of being officially named as *Glaesserella australis* sp. nov.

386

Phenotypic and genotypic variability of antibiotic-resistant *Salmonella* Typhimurium exposed to environmental stresses

Md Jalal Uddin, Gibeom Jeon, Juhee Ahn

The therapeutic misuse and overuse of antibiotics leads to the rapid emergence of antibiotic-resistance bacteria. Antibiotic-resistant bacteria can induce cross-protection against environmental stresses, resulting in the acquisition and spread of multiple antibiotic resistance. Therefore, this study aimed to assess the resistance phenotype and genotype of cefotaxime- and ciprofloxacin-induced resistant *Salmonella* Typhimurium exposed to acid, heat, and NaCl. The antibiotic susceptibilities of wild-type (WT) *S.* Typhimurium ATCC 19585 and WT *S.* Typhimurium KCCM 40253 were decreased after cefotaxime (CET) and ciprofloxacin (CIP) induction. The highest β-lactamase activities were observed at the WT and CET-induced *S.* Typhimurium CCARM 8009, showing more than 3 nmol/min/ml. The dominant FT-IR spectra at the region from 1700–1500 cm^{-1} represent proteins such as amide II and III at the CET-induced *S.* Typhimurium ATCC19585 and CIP-induced *S.* Typhimurium KCCM 4025. The CET- and CIP-induced *S.* Typhimurium strains showed better survival at pH 4.5 and 4% NaCl than the WT strains. The highest expression levels of *ompC* and *rpoS* were observed at CIP-induced *S.* Typhimurium KCCM 40253 when exposed to sublethal stresses of pH 4.5, 4% NaCl, and heat at 48^{o}C. This study provides useful information for assessing the cross-protective responses of antibiotic-resistant bacteria to environmental stresses.

1. Beceiro A, Tomás M, Bou G (2013) Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clin Microbiol Rev 26:185-230.
2. Dahiya S et al. (2014) Induction of resistant mutants of Salmonella enterica serotype Typhi under ciprofloxacin selective pressure. Indian J Med Res 139:746-753.
3. Kim J, Ahn J (2017) Characterization of clinically isolated antibiotic-resistant Salmonella Typhimurium exposed to subinhibitory concentrations of ceftriaxone and ciprofloxacin Microb Drug Resist 23:949-957.
4. Kim J, Jo A, Ding T, Lee H-Y, Ahn J (2016) Assessment of altered binding specificity of bacteriophage for ciprofloxacin-induced antibiotic-resistant Salmonella Typhimurium. Arch Microbiol 198:521-529.

387

Characterization of antibiotic-resistant *Staphylococcus aureus* exposed to oxacillin and ciprofloxacin

Md Jalal Uddin[1], Gibeom Jeon[1], Juhee Ahn[1]

1. Kangwon National University, Chuncheon, GANGWON, South Korea

The emergence of antibiotic-resistant bacteria has been of great concern due to the difficulty in chemotherapy. The aim of this study was to evaluate the resistance phenotypes and gene expression in wild-type and antibiotic-induced resistant *Staphylococcus aureus* exposed to oxacillin and ciprofloxacin. The wild-type *S. aureus* was highly resistant to oxacillin after the exposure to oxacillin, while the oxacillin resistance was not changed when exposed to ciprofloxacin. The resistance of oxacillin- and ciprofloxacin-induced *S. aureus* to penicillin was resulted from the production of β-lactamase, corresponding to the overexpression of *blaZ* (>2-fold). The efflux pump-related genes (*norA*, *norB*, *norC*, *mdeA*, *mepR*, *mgrA*, and *lmrS*) were overexpressed in oxacillin- and ciprofloxacin-induced *S. aureus*, leading to the increase in the resistance to aminoglycosides and quinolones. It is worth noting the relationship between resistance phenotype and resistance genotype in terms of antibiotic susceptibility and differential gene expression.

1. Andersson, D.I. and Hughes, D. (2010) Antibiotic resistance and its cost: is it possible to reverse resistance? Nat Rev Microbiol 8, 260–271.
2. Giraud, E., Rychlik, I. and Cloeckaert, A. (2017) Antimicrobial resistance and virulence common mechanisms. Front Microbiol 8, 5–7.
3. Jo, A. and Ahn, J. (2016) Phenotypic and genotypic characterisation of multiple antibiotic-resistant Staphylococcus aureus exposed to subinhibitory levels of oxacillin and levofloxacin. BMC Microbiol 16, 170.

388

Effect of bacteriophage-antibiotic combination on the reduction of the development of antibiotic resistance in *Salmonella* Typhimurium

Md Jalal Uddin[1], Juhee Ahn[1]

1. Kangwon National University, Chuncheon, GANGWON, South Korea

Recently, bacteriophages have been renewed attention as bactericidal agents due to their specificity to the target bacteria and no harmful effects. This study aimed to investigate the effect of bacteriophage-antibiotic combination against *Salmonella* Typhimurium and also evaluate the development of antibiotic resistance in *Salmonella* Typhimurium when treated with bacteriophage-antibiotic combination. The susceptibilities of *S.* Typhimurium to

cefotaxime, chloramphenicol, ciprofloxacin, erythromycin, streptomycin, and tetracycline were increased by 13%, 10%, 22%, 25%, 9%, and 12% in clear zone sizes, respectively, in the presence of bacteriophage P22. Minimum inhibitory concentrations (MICs) were decreased in the presence of bacteriophage P22, showing cefotaxime (0.06 to 0.03 mg/mL), chloramphenicol (4 to 0.25 mg/mL), ciprofloxacin (0.016 to 0.008 mg/mL), erythromycin (64 to 8 mg/mL), streptomycin (64 to 16 mg/mL), and tetracycline (2 to 1 mg/mL). The number of *S*. Typhimurium treated with the bacteriophage-antibiotic combination was significantly reduced by more than 5-log after 12-h of incubation at 37°C. The combination treatments could reduce the development of antibiotic resistance in *S*. Typhimurium compared to the control and single treatment (bacteriophage or antibiotic alone). The application of bacteriophages combined with antibiotic can be a promising strategy to control antibiotic-resistant bacteria. This study sheds light on possibility of using bacteriophage-antibiotic combination as therapeutic agents.

1. Bielke L, Higgins S, Donoghue A, Donoghue D, Hargis BM (2007) Salmonella host range of bacteriophages that infect multiple genera. Poult Sci 86:2536-2540.
2. Oechslin F, Piccardi P, Mancini S, Gabard J, Moreillon P, Entenza JM, Resch G, Que Y-A (2017) Synergistic interaction between phage therapy and antibiotics clears Pseudomonas aeruginosa infection in endocarditis and reduces virulence. The Journal of Infectious Diseases 215:703-712.
3. Ryan EM, Alkawareek MY, Donnelly RF, Gilmore BF (2012) Synergistic phage-antibiotic combinations for the control of Escherichia coli biofilms in vitro. FEMS Immunol Med Microbiol 65:395-398.

389

Development of indigenous novel climate resilient microbial consortium for enhancing vegetable production

Jay Prakash Verma[2, 1], Ananad Kumar Gaurav[2], Durgesh Kumar Jaiswal [2], Arthur P. Pereira [3]

1. Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, , Sydney,, NSW, Australia

2. Institute of Environment and Sustainable Development, Banaras Hindu University, India, VARANASI, UTTAR PRADESH, India

3. Department of Soil Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil., Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, São Paulo,, Brazil

In the present study, different rhizosphere soil samples of vegetable fields were collected from Varanasi region and soil microbes were isolated. Total 50 isolates were screened. Out of 50 strains, 14 strains have screened and characterized on the basis of morphological, biochemical and plant growth promoting activities. Five effective isolated strains (IESDJP-DIC3, IESDJP-DIC4, IESDJP-DIC7, IESDJP-DIC8 and IESDJP-DIC12) and two known microbial strains (*Azosprillum brasilense* IARI-1 and *Paenibacillus polymyxa* BHUPSB17) culture were selected on the basis of PGPR properties. The thirty treatment combinations were prepared with seven microbial culture. The seasonal vegetable like lobia (*Vigna unguiculata*) varieties kasha kanchan was selected for pot trials. The seed inoculation (5ml culture with each treatment volume and 10^8cfu/seed) of lobia with thirty treatments have been performed and recorded very good result in pot experiment regarding plant growth, branching, flowering and fruiting. The effective treatment combination T3, T10, T11, T13, T14, T15, T16, T17, T18, T19, T21, T22, T25, T26, T27 and T30 were found for enhancing plant growth attributes, yield, nutritional content and soil properties as compared to control and others. The microbial consortia of treatment T17 (IESDJP-DIC3 + IESDJP-DIC4 + IESDJP-DIC12), T18 (IESDJP-DIC3 + IESDJP-DIC4+ *Azosprillum brasilense)*, T19 (IESDJP-DIC3 + IESDJP-DIC4+ *Paenibacillus polymyxa*), T21 (IESDJP-DIC3 + IESDJP-DIC7+ IESDJP-DIC12), T22 (IESDJP-DIC3 + IESDJP-DIC7+ *Azosprillum brasilense*), and T27 (IESDJP-DIC3 + IESDJP-DIC8+ *Azosprillum brasilense)* have found more effective microbial consortium for labia production under sustainable agricultural practices. The indigenous climate resilient microbial consortium is a mixture of both plant growth promoting rhizobacteria and fungi. This microbial consortium will be cost-effective, environment-friendly and socially acceptable. This microbial consortium can replace 20 to 50% chemical fertilizer and pesticide application. This will improve plant growth, productivity, and nutritional quality as well as soil productivity.

390

Funcional studies of pyruvate carboxylase regulation by cyclic di-amp in lactic acid bacteria

Thu NM Vu[1], Huong T Pham[1], Raquel Lo[1], Nidhi Bansal[1], Mark Turner[1]

1. The University of Queensland, St Lucia, QLD, Australia

The broadly conserved second messenger nucleotide cyclic di-3',5'-adenosine monophosphate (c-di-AMP) has been shown to regulate a wide range of bacterial processes. C-di-AMP has been shown to bind to and allosterically regulate the activity of pyruvate carboxylase (PC) in *Listeria monocytogenes*. The c-di-AMP binding pocket residues are also conserved within PC of the economically important cheese fermentation bacterium *Lactococcus lactis*. In this work we were interested to determine the role of PC and c-di-AMP inhibition of PC in the physiology of *L. lactis*. A markerless PC deleted mutant of *L. lactis* MG1363 background (wt) and c-di-AMP insensitive PC variant overexpression mutants in a high c-di-AMP *ΔgdpP* strain (OS2) were generated and characterised. The PC deleted mutant exhibited similar growth compared to that of wt in rich media with abundant amino acids, but did not grow in chemically defined media (CDM) in the absence of aspartate or asparagine. Complementation with the wt PC gene into the PC deleted mutant restored growth in CDM. In milk, the PC deleted mutant had a significantly slower acidification rate than that of the complemented strain. Supplementation of aspartate or asparagine to milk completely restored rates of acidification and stationary-phase cell numbers of the PC deleted mutant. In the high c-di-AMP mutant strain OS2, the aspartate level was 65% lower than wt. Overexpression of c-di-AMP insensitive Y715T PC variant restored high aspartate levels comparable to that in the wt. In conclusion PC is essential for aspartate biosynthesis in *L. lactis* and is required for efficient acidification of milk, a key property of starter cultures. C-di-AMP negatively regulates the aspartate pool in *L. lactis*, and expression of a c-di-AMP insensitive LIPC can restore normal levels of aspartate.

391

Use of whole genome sequencing to determine the genetic basis of multidrug resistance in *Escherichia coli* isolated from Australian livestock.

David L Wakeham[2, 1], Brian M Forde[1], Melinda M Ashcroft[1], Leah W Roberts[1], Joanne L Mollinger[3], David Jordan[4], Nikki Buller[5], Sam Abraham[6, 7], Darren J Trott[6], Mark A Schembri[1], Justine S Gibson[2], Scott A Beatson[1]

1. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia

2. School of Veterinary Science, University of Queensland, Gatton, QLD, Australia

3. Biosecurity Sciences Laboratory, Department of Agriculture and Fisheries, Brisbane, QLD, Australia

4. New South Wales Department of Primary Industries, Wollongbar, NSW, Australia

5. Department of Agriculture & Food, South Perth, WA, Australia

6. School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia

7. School of Veterinary and Life Sciences, Murdoch University, Murdoch, WA, Australia

Introduction: The development of rapid diagnostics is key to combating multidrug-resistant (MDR) Enterobacteriaceae. Whole genome sequencing (WGS) offers rapid genetic characterisation of MDR bacteria, however, prediction of antimicrobial resistance (AMR) phenotype of Enterobacteriaceae from WGS data remains a challenge. Here we investigate the genetic diversity of MDR clinical *Escherichia coli* isolates from Australian livestock and assess the phenotypic predictive capabilities of WGS.

Methods: 44 *E. coli* isolates with multidrug resistance and/or resistance to extended-spectrum-cephalosporins (ESC) were selected from 324 isolates collected between 2013-2014 based on a weighted score from Vitek® minimum inhibitory concentration assays. Further phenotypic detection tests were performed on all suspect ESC resistant isolates. *In silico* analysis of Illumina reads and assembled genome data were used to determine the sequence type (ST), AMR genotype and virulence gene profile of each isolate.

Results: Reliable prediction of AMR phenotype from genotype using WGS for tetracyclines, aminoglycosides and folate-pathway inhibitors was shown. Sensitivity values for predictive power was greater than 0.96 for all drugs tested with the exceptions of amoxicillin-clauvanic acid and ESCs. ESC resistance could be predicted in 7 isolates by extended spectrum or *ampC* beta-lactamase carriage: $bla_{CTX\text{-}M\text{-}14}$ (n=3), $bla_{CTX\text{-}M\text{-}9}$ (n=1), or $bla_{CMY\text{-}2\text{-}like}$ (n=3), respectively. Of 22 ESC-resistant isolates (cefpodoxime ≥8 mg/L) that did not carry an extended spectrum or *ampC* beta-lactamase gene, 15 harboured mutations within the chromosomal *ampC* promotor region that has been shown to cause AmpC hyperproduction. We also identified a new *ampC* promoter mutation and a suspected insertion sequence-mediated *ampC* induction. In four cases, we could not predict the ESC phenotype from genotype.

392

Could environmental durability act as an enabler of bacterial pathogenicity?

Liang Wang[1, 2], Yangguang Du[3], Jiawei Yan[4], Qinghua Liu[2], Zhanzhong Liu[4], Charlene M Kahler[5], Michael J Wise[5, 6]

1. Department of Bioinformatics, Xuzhou Medical University, Xuzhou, Jiangsu Province, China

2. Key Laboratory of New Drug Research and Clinical Pharmacy of Jiangsu Province, Xuzhou Medical University, Xuzhou, Jiangsu Province, China

3. Xuzhou Center for Disease Control and Prevention, Xuzhou, Jiangsu Province, China

4. Department of Laboratory Medicine, Xuzhou Infectious Diseases Hospital, Xuzhou, Jiangsu Province, China

5. The Marshall Centre for Infectious Diseases Research and Training, University of Western Australia, Perth, WA, Australia

6. Computer Science and Software Engineering, University of Western Australia, Perth, WA, Australia

Sit-and-wait hypothesis predicts that environmental durability is positively correlated with bacterial virulence for non-vector-borne pathogens, such as *Mycobacterium tuberculosis* and *Pseudomonas aeruginosa*, *etc*. A couple of recent studies confirmed that environmental durability plays important roles in microbial virulence, facilitating enhanced infection rates and increased virulence for *Sphaerothecum destruens* and *Flavobacterium columnare*, respectively [1, 2]. Søborg *et al.* noticed that virulence genes are widely distributed in environmental metagenomes, indicating that virulence factors might have advantages for bacterial survival outside the host environment [3]. However, proposal of the hypothesis is merely based on the epidemiological data reported by Walther and Ewald [4]. In addition, it is argued that sit-and-wait pathogens are rather rare in microbes. In order to understand how bacterial durability and virulence are correlated, Wang *et al.* investigated hundreds of bacterial proteomes through bioinformatics methods for durability- and virulence-related factors at the genomic level [5, 6]. Energy reserves such as glycogen and polyphosphate were included as indicators of durability for the preliminary studies [6, 7]. Results showed that bacteria with energy reserve metabolism are more likely to be durable in the environment. They also have more pathogenicity-related proteins, such as invasins and toxins *etc.*, reflecting higher degree of virulence [7]. A further, detailed bioinformatics analysis into eight selected sit-and-wait pathogens confirmed that these species are tightly associated with high numbers of durability genes and virulence factors [5]. In contrast, three other bacterial categories, which are host-associated, obligate intracellular, and free-living, showed absence of durability genes or virulence factors [5]. Intra-group comparisons revealed significant differences between sit-and-wait pathogens and the other three groups [5]. The results of our bioinformatics analyses correspond well with epidemiological data presented previously [4]. Although the conclusions must be treat with caution due to limited experimental data, this preliminary study provides protein targets for investigating the sit-and-wait hypothesis and molecular experimental studies should be performed in order to provide evidence at the cellular and population levels.

1. Sana S, Hardouin EA, Gozlan RE, Ercan D, Tarkan AS, Zhang T et al. Origin and invasion of the emerging infectious pathogen Sphaerothecum destruens. Emerg Microbes Infect 6(8), e76 (2017).
2. Sundberg LR, Kunttu HM, Valtonen ET. Starvation can diversify the population structure and virulence strategies of an environmentally transmitting fish pathogen. BMC Microbiol 14 67 (2014).
3. Soborg DA, Hendriksen NB, Kilian M, Christensen JH, Kroer N. Bacterial Human Virulence Genes across Diverse Habitats As Assessed by In silico Analysis of Environmental Metagenomes. Front Microbiol 7 1712 (2016).

4. Walther BA, Ewald PW. Pathogen survival in the external environment and the evolution of virulence. Biol Rev Camb Philos Soc 79(4), 849-869 (2004).
5. Wang L, Liu Z, Dai S, Yan J, Wise MJ. The Sit-and-Wait Hypothesis in Bacterial Pathogens: A Theoretical Study of Durability and Virulence. Front Microbiol 8 2167 (2017).
6. Wang L, Wise MJ. Glycogen with short average chain length enhances bacterial durability. Naturwissenschaften 98(9), 719-729 (2011).
7. Wang L, Yan J, Wise MJ, Liu Q, Asenso J, Huang Y et al. Inorganic Polyphosphate Is Essential for Salmonella Typhimurium Virulence and Survival in Dictyostelium discoideum. Front Microbiol doi:https://doi.org/10.3389/fmicb.2018.00782 (2018).

393

Evaluation of cytotoxicity levels and synergistic antimicrobial activity of *Alpiniamalaccensis*and *Terminaliacatappa* extracts against food borne or spoilage bacteria in vacuum packed ready-to-cook (RTC) chicken

Thikshani Somarathna [1], KKDS Ranaweera [1], GAS Premakumara[2], AM Akbarsha[3], B Kadalamani[4], Nimsha S Weerakkody[5]

1. Department of Food Science and Technology, Faculty of Graduate Studies, University of Sri Jayewardenepura, Nugegoda , Sri Lanka
2. Industrial Technology Institutes, 396, Bauddhaloka Mw, Colombo, Sri Lanka
3. Mahatma Gandhi-Doerenkamp Center,, Bharathidasan University, , Tiruchirappalli 620024, , India
4. UGC National Centre for Alternatives to Animal Experiments, , , Bharathidasan University, Tiruchirappalli, , India,
5. Agicultural and Planataion Engineering , The Open University of Sri Lanka, Nawala, Colombo, Sri Lanka

Cytotoxicity levels and synergistic antimicrobial activity of *Alpinia malaccensis* and *Terminalia catappa* extracts were evaluated against *Listeria monocytogenes*, *Stapylococcus aureus* and spoilage bacteria in vacuum packed ready-to-cook chicken. Both extracts cytotoxicity levels were evaluated using human liver hepatocarcinoma cell (HepG2), normal mouse fibroblast cells (3T3) and Monkey kidney cell (COS7) by MTT assay. Chicken 10 g pieces were inoculated with each bacterium and marinated with0.5 ml of a mixture having of 5 mg/ml *A. malaccensis* and 20 mg/ml *T. catappa* extracts and vacuum packed after adding 1 ml of extract combination and stored at 4^{o}C or 8^{o}C for 12 days. Lipid oxidation and microbial counts were enumerated every three-day intervals. NoObserved Adverse Effect Concentration of *A. malaccensis* was 1.4, 30 and 1.4 µg/ml while *T. catappa* was300, 300 and 130µg/ml, for HepG2, 3T3 and COS7, respectively and calculated approximately Annual Dietary Intake values for *A. malaccensis* and *T. catappa* were 55.41g/day and 1549.70 mg/day. Marinated chicken 73.88 g is safe for consumption without any adverse health effect.Plant extracts significantly ($p<0.05$) inhibited the growth of *S. aureus* bacteria with 1.80, 2.13, 2.36 and 2.97 log cfu/g reduction over 3, 6, 9 and 12 days stored at 8 ^{o}C. Similarly, *L. monocytogenes* significantly inhibited 1.22, 1.60 and 1.55 log cfu/g reduction except on day 3. Both temperatures significantly reduced ($P<0.05$) lipid oxidation in treated chicken compared to control by 1.07 and 1.39 MDA mg/kg chicken at day 3,12 at 4 ^{o}C and 1.62, 2.35 and 2.43 MDA mg/kg during 6,9,12 days at 8 ^{o}C storage. Interestingly, 6 to 9 days at 4 ^{o}C or 9 days at 8 ^{o}C of shelf life extension achieved by marinating chicken with the combination of *A. malaccensis* and *T. catappa.*We observed significant inhibition of *L. monocytogenes*or *S. aureus* and spoilage bacteria and reduced lipid oxidation in *in-vivo* study. Therefore, marinated chicken is a great economic advantage for the food industry.

394

Capsule-switching is associated with the rapid global expansion of the recently emerged fluoroquinolone-resistant *Escherichia coli* sequence type 1193 clone

Rhys T White[1, 2, 3], Brian M Forde[1, 2, 3], Melinda M Ashcroft[1, 2, 3], Leah W Roberts[1, 2, 3], Darren J Trott[4], Justine S Gibson[5], Joanne L Mollinger[5, 6], Benjamin A Rogers[7], Amanda Kidsley[5], Jan Bell[5], John Turnidge[5], Mark A Schembri[1, 3], Scott A Beatson[1, 2, 3]

1. School of Chemistry and Molecular Biosciences, The University of Queensland, St Lucia, QLD, Australia
2. Australian Centre for Ecogenomics, The University of Queensland, Brisbane, Queensland, Australia
3. Australian Infectious Disease Research Centre, The University of Queensland, Brisbane, Queensland, Australia
4. School of Animal and Veterinary Sciences, The University of Adelaide, Adelaide, South Australia, Australia
5. School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia
6. Department of Agriculture and Fisheries, Biosecurity Sciences Laboratory, Brisbane, Queensland, Australia
7. School of Clinical Sciences, Monash University, Clayton, Victoria, Australia

Background. Uropathogenic *Escherichia coli* (UPEC) are the primary cause of urinary tract infections (UTIs), with >360.8 million global cases in 2016[1]. UTIs have become increasingly difficult to treat due to rising antibiotic resistance. Sequence type (ST)1193 is an emerging fluoroquinolone-resistant UPEC lineage increasingly associated with UTIs. Despite its clinical significance, all previous investigations of ST1193 have lacked high-resolution genomic analyses obtainable through whole-genome sequencing (WGS).

Methods. Here, through WGS and temporal phylogenomic analysis of 55 Australian ST1193 isolates spanning 2007–2013, combined with high-quality draft genomes of 50 publicly available ST1193 assemblies, we provide the most comprehensive phylogenomic characterisation of the ST1193 lineage to date. Furthermore, by generating a complete genome we have been able to characterise mobile genetic elements (MGE), allowing analysis of virulence and resistance profiles across the lineage.

Results. Our data report the earliest association of ST1193 with UTIs. Phylogenomic characterisation clusters global ST1193 isolates into two distinct clades , where clade 1 is geographically isolated to Australia. We show that ST1193 isolates from Australia are highly similar at the genomic level, however key inter-clade differences include a switch from K5 to K1 capsular antigen. The K1 capsule is associated with increased serum resistance and may be associated with the global expansion of clade 2. The majority (92.7%) of ST1193 in this study are multidrug-resistant, with eight isolates being resistant to third-generation cephalosporins following acquisition of an IncI1 plasmid carrying the *β*-lactamase genes *bla*$_{\text{CTX-M-14}}$ (*n*=1), *bla*$_{\text{CTX-M-15}}$ (*n*=4), and *bla*$_{\text{CTX-M-55}}$ (*n*=3).

Conclusion. This work represents the first comprehensive genomic characterisation of ST1193, a recently emerged fluoroquinolone-resistant UPEC clonal lineage. Our work highlights an important difference in the capsule locus within a single UPEC clone, likely due to recombination.

1. GBD Collaborative Network. Global Burden of Disease Study 2016 (GBD 2016) Results. Seattle, United States: Institute for Health Metrics and Evaluation (IHME). Available from http://ghdx.healthdata.org/gbd-results-tool (2017).

395

Soil and the gut microbiota- Supporting the “hygiene hypothesis”

Gal Winter

Background: According to the “hygiene hypothesis” the current rise in allergies and autoimmune diseases stems mainly from reduced microbial exposure due, amongst other factors, to urbanisation and distance from soil. However, this hypothesis is based on epidemiological and not biological data. Useful insights into the underlying mechanisms of this hypothesis can be gained by studying our interaction with soil. Soil microbiota may be directly ingested or inhaled by humans, enter the body through skin-soil contact or using plants as vectors. This study aims to examine the ability of soil microbiota to colonise the gut, study the interaction of soil microbes with the immune system and their potential protective activity.

Method: The nutrition of the rats was supplemented daily with fresh or autoclaved soil for 21 days followed by 14 days of no supplementations. Faecal samples were collected throughout and analysed using 16S sequencing. At the end of the experiment rats were sacrificed and tissues and digesta were collected.

Results/Conclusion: Results showed significantly higher richness and diversity following soil supplementation even after recovery. Specific soil microbial groups identified as able to colonise the gut. Of particular interest was the mucosal layer which emerged as a receptive host for soil microorganisms. Histological examination revealed innate and adaptive immune activation.

Findings of this study reinforce the “hygiene hypothesis” by demonstrating the ability of soil microbes to colonise the gut and activate the immune system. This paves the way for further studies aimed to examine the interaction of soil microorganisms with the immune system.

396

Evaluation of the Vitek 2 AST YS08 Yeast Susceptibility Test in comparison to the Sensititre YeastONE Susceptibility System for antifungal susceptibility testing

Ka Yan Wong[1], Dianne Gardam[1], Peter Boan[1]

1. Department of Microbiology, Fiona Stanley Hospital, Pathwest Laboratory Medicine WA, Perth, WA, Australia

Background:

Invasive candidiasis results in high morbidity and mortality especially in immunocompromised patients. Having an early and accurate antifungal susceptibility result is important for appropriate patient treatment. Sensititre YeastONE has good concordance with the gold standard CLSI reference method for Candida susceptibility testing and is therefore a widely utilised commercial method of determining Candida susceptibility. The automated antifungal susceptibility testing of yeasts by Vitek 2 AST YS08 has clear advantages in decreased turn-around time, reduced costs and ease of use. However, there is limited data regarding its performance.

Aim:

The purpose of the study was to evaluate the latest Vitek 2 AST YS08 yeast antifungal susceptibility card in comparison to the Sensititre YeastONE Y010 susceptibility system for antifungal susceptibility testing.

Methods:

A total of 68 clinical isolates of *Candida species*, many known to have antifungal resistance, included *Candida albicans* (20), *Candida glabrata* (21), *Candida tropicalis* (9)*, Candida parapsilosis* (10) and *Candida krusei* (8), were tested by Vitek 2 AST YS08 in comparison to the commercial Sensititre YeastONE method. Essential agreement (EA) was defined as ≤2 dilution difference and categorical agreement (CA) according to CLSI breakpoints or Epidemiological CutoffValues (ECV) as appropriate. EA and CA were determined for the antifungals Amphotericin B, Caspofungin, Micafungin, Flucytosine, Fluconazole and Voriconazole. Vitek 2 AST YS08 does not provide results for *Candida glabrata* against Fluconazole and Voriconazole, nor for *Candida krusei* against Fluconazole. There is no CLSI breakpoint or ECV for Flucystosine, therefore no CA was calculated.

Results:

Fluconazole (n=39) EA 77%, CA 90%, Voriconazole (n=47) EA 79%, CA 66%, Caspofungin (n=62) EA 100%, CA 82%, Micafungin (n=54) EA 100%, CA 100%, Amphotericin B (n=67) EA 100%, CA 100%, Flucytosine (n=68) EA 99%.

Conclusion:

In this study skewed for resistant Candida isolates, EA and CA for Vitek 2 AST YS08 and Sensititre YeastONE were suboptimal. More validation data needs to be obtained for the Vitek 2 AST YS08.

1. CLSI. Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 2nd ed. CLSI supplement M59. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
2. CLSI. Epidemiological Cutoff Values for Antifungal Susceptibility Testing. 2nd ed. CLSI supplement M60. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

397

Functional Diversity of Toxin-Antitoxin Systems in Antibiotic Resistance Plasmids in *Enterobacteriaceae*

Alma Wu[1], Muhammad Kamruzzaman[1], Jon Iredell[1, 2]

1. Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, Westmead, NSW, Australia

2. Westmead Hospital, Westmead, NSW, Australia

Introduction

The rise of transmissable antibiotic resistance (AbR) in the bacterial family *Enterobacteriaceae*, particularly *Escherichia coli* and *Klebsiella pneumoniae*, is of major concern. In these bacteria, resistance is mainly spread by self-transmissible plasmids, which commonly contain toxin-antitoxin systems (TAS). TAS ensure the stable maintenance of plasmids in bacteria by killing plasmid free cells, and contribute to the spread of AbR in and within species. This study aimed to define the TAS present in plasmids found in *K. pneumoniae*, and to determine the functions of several common TAS in various *Enterobacteriaceae* species.

Methods

TAS in plasmids found in *K. pneumoniae* were determined bioinformatically using TA Finder, and the promoter regions of TAS from different species compared. Promoter-GFP reporter systems were used to determine promoter strengths in normal growth conditions, as well as expression changes during environmental stress. Cell survival assays were also used to confirm TAS roles in stress response.

Results

A total of fifteen different TAS were identified among 306 *K. pneumoniae* plasmids, with two (*ccdAB* and *pemIK*) also being common in other *Enterobacteriaceae* plasmids. The promoters of these two TAS taken from various species and plasmids types showed variation in strength depending on the strain it was present in rather than the species. Neither plasmid borne *ccdAB* nor *pemIK* responded to nutrient limitation or antibiotic stress, nor did they confer any survival advantage under antibiotic stress.

Conclusion

The prevalence of common TAS varies between species and/or plasmid types, with some being relatively specific. TAS common to multiple plasmids vary in promoter sequence, and their expression varies between host strains, not species. Plasmid borne *ccdAB* and *pemIK* are likely to be specialised plasmid maintenance systems, and do not play a role in bacterial stress response.

398

The molecular epidemiology of an atypical wooden tongue outbreak

Sarah Yee, Bruce Jackson, Vic Epstein, Pat Blackall, Conny Turni

Wooden tongue (actinobacillosis), a sporadic disease of cattle, is caused by *Actinobacillus lignieresii*. The most prominent signs are pyogranulomatous lesions that affect the tongue and cause the tongue to become fibrous, shrunken and immobile. However, the pyogranulomatous lesions can also be found in other soft tissues such as the lymph nodes.

This case study reports an unusual outbreak of cattle of several herds affected by actinobacillosis affecting skin and lymph nodes. Nine isolates of *A. lignieresii* were isolated from the lymph nodes and identified by molecular analysis. A PCR for *A. pleuropneumoniae* was performed to exclude this species, as both species are indistinguishable by 16S rDNA gene analysis. An enterobacterial repetitive insertion consensus sequence PCR was used for genotyping and the repeats-in-toxin (RTX) genes were analysed by sequencing.

Five isolates from one outbreak on one farm shared the same genotype, which was distinctly different from the other isolates collected from different farms. A total of four genotypes were observed. All isolates contained the apxICABD operon. However, difference in the size of the *apxIA* and *apxID* genes were observed. The sequence alignment revealed that two isolates had a deletion of around 697 bp at about 736 bp into the *apxIA* gene. There was also a deletion of around187 bp at the beginning of the *apxID* gene.

The deletion and the ERIC PCR patterns suggested that several outbreaks occurred involving different *A. lignieresii* genotypes. The study provided no evidence of a unique virulence type associated with the *A. lignieresii* isolates. In turn, this suggests that the outbreaks were possibly due to a range of contributing factors, including environmental factors.

399

Structural study of a molybdenum cofactor-dependent enzyme

Sung-il Yoon[1]

1. Kangwon National University, Chuncheon, KANGWONDO, South Korea

The molybdenum cofactor (Moco) sulfurase C-terminal domain (MOSC) family plays a key role in the detoxification of mutagenic chemicals and the activation of prodrugs by catalyzing a nitrogen-reducing reaction. MOSC proteins are functionally required to bind Moco for their enzymatic reactions. However, the structural features and Moco-recognition mechanism of the MOSC family have not been revealed in detail. YiiM belongs to the MOSC family and is involved in reducing mutagenic 6-N-hydroxylaminopurine to yield nontoxic adenine in bacteria. Here, we report the crystal structure of YiiM. YiiM consists of three domains (a β-barrel and two α-helix bundles) that collectively generate a cavity in the center of the structure. Structural features critical for Moco-mediated enzymatic catalysis were observed in the cavity, including an oxidized invariant cysteine residue and positive electrostatic potentials. Furthermore, our modeling study, combined with our observation of a phosphate ion that emulates a part of a Moco molecule in the cavity, strongly supports the cavity of YiiM as the Moco-binding site where catalysis occurs.

Poster Abstracts

400

Growth stage of *Helicobacter pylori* regulates the production and content of outer membrane vesicles.

Lauren Zavan[1], Natalie J Bitto[1], Andrew F Hill[2, 3], David Greening[2, 3], Maria Liaskos[1, 3]

1. Department of Physiology, Anatomy and Microbiology, La Trobe University, Bundoora, Victoria, Australia

2. Department of Biochemistry and Genetics, La Trobe Institute for Molecular Sciences, La Trobe University, Bundoora, Victoria, Australia

3. Research Centre for Extracellular Vesicles, La Trobe University, Bundoora, Victoria, Australia

Outer membrane vesicles (OMVs) are produced by all Gramnegative bacteria and have a major role contributing to gene transfer, celltocell communication and hostpathogen interactions. Although numerous studies have examined the composition of OMVs, the impact of bacterial growth stage on regulating the size, composition and functions of OMVs has not been determined. This study aims to compare the quantity, size and composition of OMVs produced during various stages of bacterial growth.

In this study, we examined the production and composition of OMVs produced by *Helicobacter pylori* at early log, late log and stationary phase of growth (16, 48 and 72 hours of growth, respectively). We found that *H. pylori* produced approximately 1000 OMVs per bacterium during early log phase, which further increased 30-fold at 48 hrs and 32-fold at 72 hrs of bacterial growth. Furthermore, we demonstrated that OMV size decreased in heterogeneity as bacterial growth stage progressed. Specifically, OMVs from 16 hrs of growth displayed a broader range of OMV populations compared to OMVs from 48 or 72 hours of growth. Quantification of the content associated with OMVs purified from each growth stage was also determined. We found a large decrease in the amount of protein, DNA and RNA associated with OMVs as bacterial growth stage progressed from early log, to late log and stationary phase of bacterial growth. We are currently performing further analyses of OMVs to elucidate the effect of bacterial growth stage on OMV protein composition and immunogenicity.

Collectively, the findings from this study will provide detailed analysis of how bacterial growth stage affects OMV production, composition and functions. We aim to expand this study to determine if the bacterial growth stage of other Gram-negative pathogens regulates the production and content of their OMVs. Collectively, this knowledge will allow us to determine whether the regulation of OMV production and composition by bacterial growth stage is species specific or a conserved phenomenon used to increase bacterial virulence.

401

Identification of serovars' specific genes for typing the five most prevalent *Salmonella* serovars in Australia

Xiaomei Zhang[1], Michael Payne[1], Qinning Wang[2], Vitali Sintchenko[2], Ruiting Lan[1]

1. University of New South Wales, Sydney, NSW, Australia

2. Centre for Infectious Diseases and Microbiology–Public Health, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney, NSW, Australia

Background: *Salmonella enterica* is the most common cause of food-borne diseases and responsible for considerable morbidity. The case rate in Australia is 67.9 per 100,000, with 16,441 cases being notified in 2017 based on data in the National Notifiable Diseases Surveillance System (NNDSS). *S*. Typhimurium was the most frequently isolated serovar in Australia followed by *S*. Enteritidis, *S*. Virchow, *S*. Saintpaul, and *S*. infantis. There are over 120,000 *Salmonella* genomes available from public databases. In this study we used this resource to identify serovar specific genes for rapid detection of the five most commonly reported serovars in Australia.

Methods: A selected set of 2988 publicly available genome assemblies from 5 target serovars and 27 non-target serovars were analysed in this study. The genomes were annotated using PROKKA. Pan-genome and core-genome were analysed by roary. The genes unique to each target serovar were identified using in-house python scripts.

Results: STM4494, SEN1384, SESV_RS06060, SeSPB_A1749/SeSPA_A1352 and L287_11788 were determined as serovar-specific genes to Typhimurium, Enteritidis, Virchow, Saintpaul and Infantis respectively. STM4494 was absent in 2/879 Typhimurium genomes, SEN1384 was absent in 4/724 Enteritidis genomes and L287_11788 was absence in 1/107 Infantis genomes. The number of non-target serovars' genomes that contained the target gene were 8, 2, 6 to STM4494, SEN1384 and SeSPB_A1749 respectively. STM4494 located in region $tRNA^{leuX}$ was only present in Typhimurium, while SEN1384 was in the region GEI/SE14 unique to Enteritidis and SESV_RS06060 was in GI-*leuX* region only present in Virchow. Conventional PCR confirmed the specificities of these target genes.

Conclusion: Using Bioinformatic analysis, genes specific to each of five of Australia's most frequently isolated serovars were identified. These serovar specific genes are suitable for developing rapid real time PCR based serovar detection and identification.

1. Bishop AL, et al. Analysis of the hypervariable region of the Salmonella enterica genome associated with tRNA(leuX). J Bacteriol. 2005 Apr;187(7): 2469-82.
2. Agron, et al. 2001. Identification by subtractive hybridization of sequences specific for Salmonella enterica serovar Enteritidis. Appl. Environ. Microbiol. 67:4984-4991
3. CA. Santviago, et al. 2010. Spontaneous Excision of the Salmonella enterica Serovar Enteritidis-Specific Defective Prophage-Like Element φSE14. J Bacteriol. 2010 Pr; 192(8): 2246-2254.
4. Bachmann, et al. Genome analysis and CRISPR typing of Salmonella enterica serovar Virchow. BNC Genomic 2014, 15:389

402

Metabolomic analysis uncovered the synergistic mechanisms of polymyxin B in combination with rifampicin against MDR *Acinetobacter baumannii*

Jinxin Zhao[1], Yu-Wei Lin[1], Meiling Han[1], Darren Creek[2], Tony Velkov[3], Yan Zhu[1], Jian Li[1]

1. Department of Microbiology, Monash University, Melbourne, VIC, Australia

2. Drug Delivery, Disposition and Dynamics, Monash University, Melbourne, VIC, Australia

3. Department of Pharmacology and Therapeutics, University of Melbourne, Melbourne, VIC, Australia

Background:

Polymyxins are currently used as the last-line therapy against multidrug-resistant (MDR) *Acinetobacter baumannii*. As resistance to polymyxins can emerge in *A. baumannii* with monotherapy, combination therapies are often employed in the clinic. Previous studies showed that polymyxin-rifampicin combination displayed synergistic killing against MDR *A. baumannii*; however, the synergistic mechanism remains unclear. In the present study, we employed metabolomics to investigate the synergistic mechanisms of polymyxin B-rifampicin combination against a model strain of MDR *A. baumannii* AB5075.

Methods:

Bacterial log-phase culture was treated with polymyxin B (0.75 mg/L), rifampicin (1 mg/L), and their combination, respectively. Samples were collected at 0, 1, 4 and 24 hr, and LC-MS was employed to analyse the metabolome. MzMatch, IDEOM and MetaboAnalyst were used for bioinformatic analysis. Metabolites with fold change > 2, FDR < 0.05 were subjected to pathway analysis.

Results:

Polymyxin B monotherapy only caused early (1 hr) perturbation of Phosphatidylethanolamines metabolism (e.g. *sn*-glycero-3-phosphoethanolamine). Rifampicin monotherapy induced significant perturbations in nucleotide and amino acid metabolism (14 metabolites) at 4 hr. More key metabolic pathways (e.g. energy, lipid, nucleotide, amino acid metabolism) were significantly perturbed by the combination at 1 and 4 hr (36 and 61 significant metabolites, respectively). Significant changes in the levels of glycerophospholipids and fatty acids were observed after the combination treatment for 1 and 4 hr. Of particularly interested is that the combination exclusively increased the intermediate metabolite pools in pentose phosphate pathway at 1 hr. Furthermore, the pyrimidine metabolism and histidine degradation pathways were significantly increased. Interestingly, metabolites in the nucleotide and amino acid biosynthesis pathways were significantly decreased at 4 hr. Compared to each monotherapy, most key metabolic pathways were disrupted by this combination.

Conclusions:

This is the first study to employ metabolomics to unveil the synergistic killing mechanisms by polymyxin-rifampicin combination against MDR *A. baumannii*. The time-dependent synergistic activity via disruption of PPP, nucleotide and amino acid metabolism will help design better polymyxin combinations in the clinic.

403

Elucidating the Zn(II)-binding mechanism of the pneumococcal protein AdcAII

Marina L Zupan[1], Zhenyao Luo[2, 3, 4], Victoria G Pederick[1], Bostjan Kobe[2, 3, 4], James C Paton[1], Christopher A McDevitt[1]

1. Research Centre for Infectious Diseases, The University of Adelaide, Adelaide, SA, Australia

2. School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD, Australia

3. Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD, Australia

4. Institute for Molecular Bioscience, The University of Queensland, Brisbane, QLD, Australia

Streptococcus pneumoniae (the pneumococcus) is a globally significant human pathogen responsible for 1 – 2 million deaths annually. To colonise and persist within the host, the pneumococcus must acquire the transition metal ion zinc [Zn(II)], present at low concentrations in the host environment. In *S. pneumoniae*, Zn(II) import is facilitated by the ATP-binding cassette transporter, AdcCB, and two Zn(II)-specific solute binding proteins, AdcA and AdcAII. Although AdcA and AdcAII both deliver Zn(II) to the AdcCB transporter, AdcAII has a more critical role for survival during Zn(II) starvation. Despite the known importance of AdcAII in pneumococcal Zn(II) uptake, the molecular details of how the protein selectively acquires Zn(II) remain poorly understood. To date, our understanding of the Zn(II)-binding mechanism has been based solely on the Zn(II)-bound crystal structure of AdcAII, with an open, metal-free conformation remaining refractory to crystallographic approaches. As a consequence, the conformational changes that occur within AdcAII upon Zn(II)-binding remain unknown. Here, we overcame this issue by mutating each of the four Zn(II)-coordinating residues of AdcAII and performing structural and biochemical analyses on the variant isoforms. Structural analyses of the Zn(II)-bound AdcAII variant isoforms revealed how specific regions within the protein undergo conformational changes via their direct coupling to each of the metal-binding residues. Complementing this work, metal-binding studies revealed that mutagenesis of the coordinating residues altered both the metal ion selectivity of the protein and its affinity for Zn(II). Collectively, these results provide new insight into the mechanism of Zn(II)-binding by AdcAII and the biophysical basis by which the protein confers selectivity for this essential metal ion.

Poster Abstracts

404

Development of a method for *in-situ* characterisation of biofilms grown on meat muscle

Nirmani Wickramasinghe, Josh T Ravensdale, Ranil Cooray, Gary A Dykes, Scott P Chandry

Psychrotrophic *Pseudomonas* species are the dominant bacteria on aerobically stored chilled meat. Biofilm formation during chilled storage and transportation results in slime formation which is a major spoilage characteristic. Few studies have investigated biofilm formation on meat. In this work, a method was developed to investigate biofilm growth on meat using evenly sectioned sterile meat slices placed in six well plates permitting modest throughput and replication. *Pseudomonas fragi* (n=5) and *Pseudomonas lundensis* (n=5) were selected from a previously characterised collection based on their volatile production during spoilage on sterile beef. Meat samples were inoculated with cell numbers similar to those on retail meat and incubated at 4 ^{0}C and 10 ^{0}C for three, five and seven days to form mono species biofilms. At each time point, biofilms were stained with Syto 9 and Propidium Iodide to image live/dead proportions of bacteria. To observe the structure of eDNA network, TOTO 1 and Syto 60 were used on the seventh day. Two independent experimental rounds were carried out and confocal microscopy was used to obtain Z stacks which were quantified for mean biomass using biofilm analysing software COMSTAT. The structural and cellular organisation of the biofilms on muscle tissue were analysed using 3D visualization software AVIZO. The cell count data on biofilms correlated well with microscopic observations and the results were reproducible. *Pseudomonas* biofilms on sterilised beef were structurally similar to those of adventitious microorganisms on non-sterile beef slices incubated under identical conditions. Based on mean bio volume data, some *P. fragi* strains produced biofilms more rapidly than *P. lundensis* at 4^{0}C than at 10^{0}C. Highly dense, thick biofilms are a characteristic of *P. fragi* strains. The bacterial cells in *P. fragi* biofilm appeared to be vertically oriented whereas this characteristic was not observed in *P. lundensis* biofilms.

Delegate List

First	Last	Orginisation	Email
Matthew	Sullivan	Griffith University	matthew.sullivan@griffith.edu.au
Kate	Seib	Institute for Glycomics, Griffith University	k.seib@griffith.edu.au
Lucy	Furfaro	University of Western Australia	lucy.furfaro@uwa.edu.au
Ross	Barnard	University of Queensland	rossbarnard@uq.edu.au
Cheryl	Power	University of Melbourne	cheryljp@unimelb.edu.au
Rininta	Firdaus	University of Nottingham	rininta.firdaus@nottingham.ac.uk
Adam	Taylor	Griffith University	a.taylor1@griffith.edu.au
Fabian	Amalraj	International Medical University	fabian_davamani@imu.edu.my
Liang	Wang	Xuzhou Medical University	leonwang@xzhmu.edu.cn
Juhee	Ahn	Kangwon National University	juheeahn@kangwon.ac.kr
Md Jalal	Uddin	Kangwon National University	jalalmbe@kangwon.ac.kr
Gibeom	Jeon	Kangwon National University	ss01208@kangwon.ac.kr
Makrina	Totsika	Queensland University of Technology	makrina.totsika@qut.edu.au
Roy	Robins-Browne	University of Melbourne	r.browne@unimelb.edu.au
Ian	Macreadie	RMIT University	ian.macreadie@rmit.edu.au
Frances	Morey	Western Diagnostic Pathology	fmorey3@bigpond.com
Aimee	Tan	Griffith University	aimee.a.tan@gmail.com
Nimsha	Weerakkody	Open University of Sri Lanka	nweer@ou.ac.lk
Amy	Jennison	Forensic and Scientific Services, Department of Health	amy.jennison@health.qld.gov.au
Catherine	Janto	NSW Health Pathology	catherine.janto@health.nsw.gov.au
Marwa	Hassan	University of Queensland	marwa.hussainali@uq.net.au
Jessica	Lewis	Monash University	jessica.lewis@monash.edu
Emily	Gulliver	Monash University	emily.gulliver@monash.edu
Michael	Burch	Monash University	michael.burch@monash.edu
Marianne	Mégroz	Monash University	marianne.megroz@monash.edu
Tracey	Steinrucken	CSIRO	tvsteinrucken@gmail.com
Rabeb	Dhouib	QUT	rabeb.dhouib@qut.edu.au
Joanne	Connolly	Charles Sturt University	jconnolly@csu.edu.au
Liesel	Stassen	Queensland University of Technology	liesel.stassen@gmail.com
Karen	Weynberg	University of Queensland	k.weynberg@uq.edu.au
Begoña	Heras	La Trobe University	b.heras@latrobe.edu.au
Stacey	Hong	University of Western Australia	stacey.hong@research.uwa.edu.au
Muhammad Fahmi	Ismail	KK Women's and Children's Hospital	muhd.fahmi.ismail@gmail.com
Jillian	Carr	Flinders University	jill.carr@flinders.edu.au
Cheryl-Lynn Y.	Ong	University Of Queensland	y.ong@uq.edu.au
John	Dearnaley	University of Southern Queensland	john.dearnaley@usq.edu.au
April	Pottinger	Royal Hobart Hospital, Hobart, TAS	april.pottinger@ths.tas.gov.au

Delegate List

First	Last	Orginisation	Email
Joanne	Macdonald	University of the Sunshine Coast	jmacdon1@usc.edu.au
David	Wakeham	University of Queensland	d.wakeham@uq.edu.au
Dane	Lyddiard	University of New England	dlyddiar@myune.edu.au
Christopher	Day	Griffith University	c.day@griffith.edu.au
Rhys	Izuagbe	Queensland University of Technology	rhys.izuagbe@hdr.qut.edu.au
Hafiz	Iqbal	Tecnologico de Monterrey, School of Engineering and Science	hafiz.iqbal@my.westminster.ac.uk
Minh Duy	Phan	University of Queensland	m.phan1@uq.edu.au
Aroon	Supramaniam	Griffith University	aroon.supramaniam@griffithuni.edu.au
Ka Yan	Wong	Pathwest Laboratory Medicine WA	kayan_w87@hotmail.com
Erwin	Lamping	University of Otago	erwin.lamping@otago.ac.nz
Robert	Norton	Townsville Hospital	robert.norton@health.qld.gov.au
Neil	Blackwell	Hologic	neil.blackwell@hologic.com
Robyn	Marsh	Menzies School Of Health Research	robyn.marsh@menzies.edu.au
Katharina	Richter	University of Adelaide	katharina.richter@adelaide.edu.au
Su Chen	Lim	University Of Western Australia	lim_suchen@yahoo.com
Niloufar	Roshan Hesari	University of Western Australia	20972687@student.uwa.edu.au
Amy	Pham	University of Queensland	amy.pham@uq.net.au
Alma	Wu	Westmead Institute for Medical Research	almawu656@gmail.com
Ryan	Spargo	Griffith University	ryan_spargo@msn.com
Mohammad	Hamidian	University of Technology Sydney	mohammad.hamidian@uts.edu.au
Christopher	Harmer	University Of Sydney	christopher.harmer@sydney.edu.au
Evgeny	Semchenko	Griffith University	e.semchenko@griffith.edu.au
YeonGyun	Jung	Kyungpook National University	jyg1076@knu.ac.kr
Christopher	Mullally	University of Western Australia	20508933@student.uwa.edu.au
Victoria	Korolik	Griffith University	v.korolik@griffith.edu.au
Ambika	Murthy	University of Queensland	a.murthy@imb.uq.edu.au
Joanne	Devlin	University of Melbourne	devlinj@unimelb.edu.au
Huy	Pham Quang	Lab of Molecular Microbiology, Kyungpook National University, South Korea.	huypham@knu.ac.kr
Chang Eon	Park	Kyungpook National University	aeonrapt@knu.ac.kr
Divya	Dharshini	International Medical University, Malaysia	divya.dharshini@student.imu.edu.my
Meshalanirani	Mareshvaran	International Medical University (IMU) Malyasia	meshalanirani.mareshvaran@student.imu.edu.my
Karena	Waller	University Of Melbourne	klwaller@unimelb.edu.au
Lida	Omaleki	University of Queensland	omalekil@yahoo.com
Beau	Picking	National Herbarium of Victoria and The University of Melbourne	pickingb@unimelb.edu.au
Michael	Gantier	Hudson Institute of Medical Research	michael.gantier@monash.edu
Xiaomei	Zhang	University of New South Wales	amayzxm@gmail.com

Delegate List

First	Last	Orginisation	Email
Ruiting	Lan	University Of New South Wales	r.lan@unsw.edu.au
Michelle	Chamoun	Griffith University	michelle.chamoun@griffithuni.edu.au
Jack	Wang	University of Queensland	t.wang1@uq.edu.au
Daniel	Knight	Murdoch University	daniel.knight@murdoch.edu.au
Gregory	Crocetti	Scale Free Network	gregorycrocetti@yahoo.com.au
Martina	Jelocnik	University of Sunshine Coast	martina.jelocnik@research.usc.edu.au
Carola	Venturini	Westmead Institute for Medical Research	carola.venturini@gmail.com
Christopher	Rodrigues	University of Technology Sydney	christopher.rodrigues@uts.edu.au
Stephanie	Lynch	La Trobe University, Bundoora	18091121@students.latrobe.edu.au
Fiona	Sansom	University of Melbourne	fsansom@unimelb.edu.au
Terry	Mulhern	University of Melbourne	tmulhern@unimelb.edu.au
Flavia	Huygens	Queensland University Of Technology	f.huygens@qut.edu.au
Corey	Davies	Queensland University Of Technology	c12.davies@hdr.qut.edu.au
BM Chamini	Yapa	Queensland university of Technology	bmwim@ou.ac.lk
Deirdre	Collins	ECU	deirdre.collins@ecu.edu.au
Ian	Buckley	Hobart Pathology	buckley.ian@bigpond.com
Christian	Rinke	The University of Queensland	c.rinke@uq.edu.au
Megan	Lloyd	Edith Cowan University	m.lloyd@ecu.edu.au
Lisa	Stinson	University of Western Australia	lisa.stinson@uwa.edu.au
Brianna	Atto	University of Tasmania	baatto@utas.edu.au
Norvie	Aquino	RCPAQAP	norviember@yahoo.com
Wai	Leong	NTU-SCELSE	wleong011@e.ntu.edu.sg
Diane	Ouwerkerk	Department of Primary Industries and Fisheries	diane.ouwerkerk@daf.qld.gov.au
Yenkai	Lim	QUT	y44.lim@hdr.qut.edu.au
John	Atack	Griffith University	j.atack@griffith.edu.au
Deborah	Williamson	Microbiological Diagnostic Unit Public Health Laboratory	deborah.williamson@unimelb.edu.au
Jaclyn	Pearson	Hudson Institute Of Medical Research	jaclyn.pearson@hudson.org.au
Alison	Luk	University of Sydney	aluk7042@uni.sydney.edu.au
Antony	Della-Porta	Bio2ic	tony.della-porta@bio2ic.com
Nijoy	John	University of Melbourne	njohn@student.unimelb.edu.au
Mwila	Kabwe	La Trobe University	19114447@students.latrobe.edu.au
Yogitha	Srikhanta	Monash University	yogitha.srikhanta@monash.edu
Bethany	Bowring	The Westmead Institute for Medical Research	bethany.bowring@sydney.edu.au
Priscilla	Johanesen	Monash University	priscilla.johanesen@monash.edu
Arjuna	Ellepola	Faculty of Dentistry, Health Sciences Center, Kuwait University	arjuna@hsc.edu.kw
Kelly	Wyres	University of Melbourne	kelly.wyres@googlemail.com

Delegate List

First	Last	Orginisation	Email
Muhammad	Kamruzzaman	Westmead Institute for Medical Research, The University of Sydney	mkamruzzaman@sydney.edu.au
Julian	Rood	Monash University	julian.rood@monash.edu
Joanna	Musik	Griffith University	joanna.musik@griffithuni.edu.au
Mahboobeh	Behruznia	The Australian National University	mahboobeh.behruznia@anu.edu.au
Gavin	Smith	Duke-NUS Medical School	gavin.smith@duke-nus.edu.sg
Enzo	Palombo	Swinburne University Of Technology	epalombo@swin.edu.au
Margaret	Spiteri	AgEtal Pty Ltd	margaret@agetal.com.au
Aleksandra	Petrovic Fabijan	The Westmead Institute for Medical Reserach	aleksandra.petrovicfabijan@sydney.edu.au
Ammar	Aziz	Menzies School of Health Research	spidermine63@gmail.com
Andrew	Gordon	Consultant Physician	andrew.gordon.e5@webone.com.au
Alan	Heritage	Heritage & Associates	alanheritage1@yahoo.com.au
Clare	Smith	University of Massachusetts Medical School	clare.smith@umassmed.edu
Jennifer	Hosmer	University of Queensland	jennhosmer@optusnet.com.au
Daniel	Rawle	QIMR Berghofer Medical Research Institute	daniel.rawle@qimrberghofer.edu.au
Michael	Wise	University Of Western Australia	michael.wise@uwa.edu.au
Vikrant	Minhas	University of Adelaide	vikrant.minhas@adelaide.edu.au
Marufa	Nasreen	University of Queensland	marufa.nasreen@uq.net.au
Julianne	Djordjevic	Westmead Institute for Medical Research, and Sydney Medical School-Westmead University of Sydney	julianne.djordjevic@sydney.edu.au
Eloise	Stephenson	Griffith University	eloise.stephenson@griffithuni.edu.au
John	Boyce	Monash University	john.boyce@monash.edu
Pat	Blackall	University of Queensland	p.blackall@uq.edu.au
Mark	Turner	University Of Queensland	m.turner2@uq.edu.au
Rod	Givney	NSW Health Pathology	rodney.givney@hnehealth.nsw.gov.au
Dena	Lyras	Monash University	dena.lyras@monash.edu
Gary	Dykes	Curtin Univerity	gary.dykes@curtin.edu.au
Judith	Kelly	UTS	kellyfamhis@yahoo.com.au
Carole-Joy	Kidd	Pathology Queensland - Cairns	carolejkidd@gmail.com
David	Harrich	QIMR Berghofer	david.harrich@qimrberghofer.edu.au
Lauren	Zavan	La Trobe University	lazavan@students.latrobe.edu.au
Jillian	Templeton	DAFF Queensland	jillian.templeton@qld.gov.au
Buddhie	Nanayakkara	Australian National University	buddhie.nanayakkara@anu.edu.au
Thu	Vu	The University of Queensland	ngoc.vu@uqconnect.edu.au
Rosalind	Gilbert	Queensland Government	ros.gilbert@daf.qld.gov.au
Eileen	Roulis	Australian Red Cross Blood Service	eileen.roulis@gmail.com
Yvonne	Su	Duke-NUS Medical School	yvonne.su@duke-nus.edu.sg
Andrea	McWhorter	University of Adelaide	andrea.mcwhorter@adelaide.edu.au

Delegate List

First	Last	Orginisation	Email
Maria	Liaskos	La Trobe University	m.liaskos@latrobe.edu.au
Natalie	Bitto	La Trobe University	natalie.bitto@outlook.com
Paraic	O Cuiv	University of Queensland	p.ocuiv@uq.edu.au
Casey	Huang	The University of Queensland	kuantzu.huang@uqconnect.edu.au
Pongthorn	Pumtang-on	Charls Sturt Univeristy	pongthornp7@gmail.com
Timothy	Wells	University of Queensland	timothy.wells@uq.edu.au
Glen	Ulett	Griffith University	g.ulett@griffith.edu.au
Sarah	Tozer	QPID - Childrens Health Queensland	s.tozer@uq.edu.au
Cheryl	Jenkins	NSW Department of Primary Industries	cheryl.jenkins@dpi.nsw.gov.au
Seweryn	Bialasiewicz	Children's Health Queensland	seweryn@uq.edu.au
Erin	Price	University of the Sunshine Coast	esme_epp@hotmail.com
Derek	Sarovich	University of the Sunshine Coast	dsarovi1@usc.edu.au
Aidan	Kane	University of Sydney	aidan.kane@sydney.edu.au
Joshua	Monteith	University of Queensland	joshua.monteith@uq.net.au
Behnaz	Heydarchi	University of Melbourne	bheydarchi@unimelb.edu.au
Sarah	Yee	UQ QAAFI	s.yeewenyi@uq.edu.au
Thotsapol	Thomrongsuwannakij	Faculty of Veterinary Science, Chulalongkorn University	thotsapol_t@yahoo.com
Agnes	dela Cruz	The University of Queensland	agnes.delacruz@uqconnect.edu.au
Murari	Bhandari	Queensland University of Technology	murari.bhandari@hdr.qut.edu.au
Cynthia	Whitchurch	University Of Technology Sydney	cynthia.whitchurch@uts.edu.au
Conny	Turni	University of Queensland	c.turni1@uq.edu.au
Dewa Rasmika	Dewi	University of New South Wales Sydney Australia	d.dewi@student.unsw.edu.au
Lucy	Shewell	Griffith University	l.shewell@griffith.edu.au
Zahra	Islam	Monash University	zahra.islam@monash.edu
Dhruba	Acharya	Griffith University	dhruba.acharya@griffithuni.edu.au
Matthew	Payne	University of Western Australia	matthew.payne@uwa.edu.au
Yaramah	Zalucki	Griffith University	y.zalucki@griffith.edu.au
Mark	Read	University of Sydney	mark.read@sydney.edu.au
Rhys	White	The University of Queensland	rhys.white@uqconnect.edu.au
Melinda	Ashcroft	University of Queensland	melinda.ashcroft@uqconnect.edu.au
Ben	Huang	Queensland Health Forensic and Scientific Service	ben.huang@health.qld.gov.au
David	Lizarraga	University of Tasmania	david.lizarraga@utas.edu.au
Carrie	Coggon	The University of Queensland	c.coggon@uq.edu.au
Martha	Zakrzewski	QIMR Berghofer	martha.zakrzewski@qimrberghofer.edu.au
Pratibha Malini	James	NSW Health Pathology East	dr.pratibhamalini@gmail.com
Verna	Monsanto-Hearne	University of Queensland	v.hearne@uq.edu.au
Eugene	Madzokere	Griffith University	eugene.madzokere@griffithuni.edu.au

Delegate List

First	Last	Orginisation	Email
Miljan	Stupar	University of Queensland	miljan.stupar@uqconnect.edu.au
Gregory	Moseley	Monash University	greg.moseley@monash.edu
Cheuk Hei	Yim	UQ	helloyim84@gmail.com
Ella	Johnston	La Trobe University	eljohnston@students.latrobe.edu.au
Stanley	Chen	CSIRO	stanley.chen@csiro.au
Rachael	Lappan	The University of Western Australia	20939116@student.uwa.edu.au
Damian	Purcell	University of Melbourne	dfjp@unimelb.edu.au
Jayne	Manning	Murdoch Childrens Research Institute	jayne.manning@mcri.edu.au
Robert	Flower	Australian Red Cross Blood Service	rflower@redcrossblood.org.au
Huong	Pham	The University of Queensland	thi.pham13@uqconnect.edu.au
Pierre	Offre	NIOZ Royal Netherlands Institute for Sea Research and Utrecht University	pierre.offre@nioz.nl
Ed	Fox	CSIRO	edward.fox@csiro.au
Jeremy	Barr	Monash University	jeremy.barr@monash.edu
Erin	Brazel	University of Adelaide	erin.brazel@adelaide.edu.au
Clair	Joyce	Tecan Australia Pty Ltd	clair.joyce@tecan.com
Julie	Allerton	NSW Health Pathology	julie.allerton@health.nsw.gov.au
Amreeta	Sarjit	Curtin University	amreeta.sarjit@postgrad.curtin.edu.au
Angelita Lopez	McKenzie	Nambour General Hospital	abmckenzie@hotmail.com
Ian	Peak	Griffith University	i.peak@griffith.edu.au
Brenda	Govan	James Cook University	brenda.govan@jcu.edu.au
Paulina	Dzianach	Curtin University	paulina.dzianach@postgrad.curtin.edu.au
Nirmani	Wickramasinghe	Curtin University	nirmani.wickramasinghe@csiro.au
Jacqueline	Wiley	Dermcare Vet	jacqueline.wiley@dermcare.com.au
Jean	Moselen	Victorian Infectious Diseases Reference Laboratory	jean.moselen@mh.org.au
Marina	Zupan	University of Adelaide	marina.zupan@adelaide.edu.au
Diane	McDougald	University Of Technology Sydney	diane.mcdougald@uts.edu.au
Parisa	Noorian	University of Technology Sydney	paris.noorian@gmail.com
Nawal	Alsamdani	Flinders University	naanaa-cute@hotmail.com
Chiara	Carnevali	University of Queensland	c.carnevali@uq.edu.au
Simon	Villegas-Ospina	The University of Queensland	s.villegasospina@uq.edu.au
Tom	Ross	University of Tasmania	tom.ross@utas.edu.au
Alexander	Pintara	Queensland University of Technology	alexander.pintara@hdr.qut.edu.au
Sumeet	Sandhu	Queensland university of Technology	sumeet.sandhu@hdr.qut.edu.au
Amorette	Bezuidenhout	Queensland University Of Technology	amore.pretorius@hdr.qut.edu.au
Caitlin	Medley	Queensland University Of Technology	caitlin.medley@connect.qut.edu.au
Brianna	Steed	The University of Melbourne	bsteed@student.unimelb.edu.au

Delegate List

First	Last	Orginisation	Email
Vincent	Wheatley	University of Queensland	v.wheatley@uq.edu.au
Simon	Reid	The University of Queensland	simon.reid@uq.edu.au
Stephen	Graves	Australian Rickettsial Reference Laboratory	graves.rickettsia@gmail.com
Robert	Gibb	Pathology Queensland	robert.gibb@health.qld.gov.au
Gilda	Tachedjian	Burnet Institute	gildat@burnet.edu.au
Lucia	Zacchi	The University of Queensland	l.zacchi@uq.edu.au
Linda	Henderson	University of Sydney	lhen2084@uni.sydney.edu.au
Sacha	Pidot	University of Melbourne	sacha.pidot@unimelb.edu.au
Andrew	Lawrence	SA Pathology	andrew.lawrence@health.sa.gov.au
Suhui	Zhao	The University of Hong Kong	suhui@connect.hku.hk
Nola	Hitchick	Douglass Hanly Moir	nhitchick@dhm.com.au
Sian	Pottenger	University of Queensland	s.pottenger@uq.edu.au
Mike	Mason	University of Queensland	michael.mason@uq.edu.au
Ella	Trembizki	University of Queensland	e.trembizki@uq.edu.au
Abdulrahman	Almohammed	University of Sydney	aalm2019@uni.sydney.edu.au
Justin	Seymour	University Of Technology Sydney	justin.seymour@uts.edu.au
Johanna	Kenyon	Queensland University of Technology	johanna.kenyon@sydney.edu.au
Rob	Edwards	San Diego State University	redwards@mail.sdsu.edu
Patrick	Harris	University of Queensland	padstock@hotmail.com
Louise	Roddam	University Of Tasmania	louise.roddam@utas.edu.au
Jai	Tree	University of New South Wales	j.tree@unsw.edu.au
Nicky	Thomas	University of South Australia	nicky.thomas@unisa.edu.au
Dabah	Moukachar	SA Pathology - Flinders Medical Centre	dabah.moukachar@sa.gov.au
Michael	Doane	San Diego State University	mpdoane2@gmail.com
Harriet	Whiley	Flinders University	harriet.whiley@flinders.edu.au
Anita	Williams	Biomerieux Australia	anita.williams@icloud.com
Chris	Huang	Phagelux Australia Pty Ltd	xchrish@live.com
Erandi	Pathirana	The University of Sydney	bhagini.pathirana@sydney.edu.au
Natalie	Spillman	University of Melbourne	natalie.spillman@unimelb.edu.au
Su Hung	Ching	PROGEL Pty Ltd and PERKii Pty Ltd	s.ching@uq.edu.au
Rytas	Vilgalys	Duke University	fungi@duke.edu
Barbara	Butow	Food Standards Australia New Zealand	barbara.butow@foodstandards.gov.au
Aaron	Jex	The Walter and Eliza Hall Institute of Medical Research	jex.a@wehi.edu.au
Charlene	Kahler	University Of Western Australia	charlene.kahler@uwa.edu.au
Karen	Kong	City University of Hong Kong	khk.karen@gmail.com
Heroen	Verbruggen	University of Melbourne	heroen.verbruggen@gmail.com
Vi Khanh	Truong	Swinburne University of Technology	vtruong@swin.edu.au

Delegate List

First	Last	Orginisation	Email
Stuart	Denman	CSIRO	stuart.denman@csiro.au
Timothy	Kidd	The University of Queensland	t.m.kidd@uq.edu.au
Jamie	Triccas	University Of Sydney	j.triccas@usyd.edu.au
Charlotte	Oskam	Murdoch University	c.oskam@murdoch.edu.au
Stephanie	Owen	Swinburne University of Technology	stephaniejag@hotmail.com
Peter	Buchanan	Landcare Research	buchananp@landcareresearch.co.nz
Patrick	Reading	Peter Doherty Institute for Infection and Immunity	preading@unimelb.edu.au
Ben	Schulz	University of Queensland	b.schulz@uq.edu.au
Jenny	Evans	Sullivan Nicolaides Pathology	jenny_evans@snp.com.au
Lesley	Duffy	CSIRO	lesley.duffy@csiro.au
Stephen	Barker	University of Queensland	s.barker@uq.edu.au
Dorothy	Machalek	The Royal Women's Hospital	dorothy.machalek@mcri.edu.au
Gal	Winter	University of New England	gwinterz@une.edu.au
Kyra	Cottrell	University of Queensland	k.cottrell@uq.edu.au
Kumar	Selvarajoo	Agency for Science, Technology & Research	kumar_selvarajoo@biotrans.a-star.edu.sg
Tim	Stinear	University of Melbourne	tstinear@unimelb.edu.au
Winkie	Fong	Centre of Infectious Diseases and Microbiology - Public Health	winkie.fong@hotmail.com
Nway	Mone	The University of Queensland	n.mone@uq.edu.au
Verlaine	Timms	Centre of Infectious Diseases - Public Health	verlaine.timms@health.nsw.gov.au
Cameron	Buckley	UQ Centre for Clinical Research	c.buckley2@uq.edu.au
Rachel	Thomson	Greenslopes Private Hospital	r.thomson@uq.edu.au
Michael	McDonald	Monash University	mike.mcdonald@monash.edu
Munazza	Rajput	Australian National University	munaleo@hotmail.com
Merrin	Tulloch	MSD	merrin.tulloch@merck.com
Erin	Shanahan	The University of Sydney	erin.shanahan@sydney.edu.au
Miranda	Pitt	University of Queensland	miranda.pitt@uq.edu.au
Shabana	Shabana	Department of Botany, University of the Punjab	shabanawagi@gmail.com
Brian	Forde	University of Queensland	b.forde@uq.edu.au
Samuel Dominggus Chandra	Siahaan	Faculty of Medicine Universitas Indonesia	samueldcs.94@gmail.com
Megan	Petterson	Manaaki Whenua - Landcare Research	pettersonm@landcareresearch.co.nz
Seong San	Kang	Curtin University and CSIRO	sanga.kang@csiro.au
Anggia	Prasetyoputri	The University of Queensland	a.prasetyoputri@uq.edu.au
Behrad	Radmehr	Swinburne University Of Technology	bradmehr@swin.edu.au
Shakeel	Mowlaboccus	University of Western Australia	shakeel_mowlaboccus@hotmail.com
Jinxin	Zhao	Monash University	jinxin.zhao@monash.edu
Su	Aye	Monash University	su.aye@monash.edu

Delegate List

First	Last	Orginisation	Email
Greg	Ashworth	Cell Biosciences Pty Ltd	greg@cellbiosciences.com.au
Kalliopi	Kazanis	Cell Biosciences	kalliopi@cellbiosciences.com.au
Josh	Ravensdale	Curtin University	joshua.ravensdale@curtin.edu.au
Kyeong Ah	Lee	Korea Center for Disease Control and Prevention	leeka0320@korea.kr
Joanne	Meers	University of Queensland	j.meers@uq.edu.au
Seon Do	Hwang	Korea Center for Disease Control and Prevention	hwangsd@korea.kr
Andrea	Bustamante	NSW Health Pathology- Westmead Hospital	andrea.bustamante@health.nsw.gov.au
Rifky	Balgahom	NSW Health Pathology - Nepean	rifky.balgahom@gmail.com
Paul	Bartley	Wesley Private Hospital	pbartley@wesley.com.au
Andrew	Szentirmay	Gene Target Solutions	pip@genetargetsolutions.com
Gareth	Price	Queensland Facility of Advanced Bioinformatics	g.price@qfab.org
Fallen	Teoh	Macquarie University	fallen.teoh@students.mq.edu.au
Rohininathan	Gopalsamy	LIFE SCIENCE RESOURCE CENTRE	rohininathan1965@yahoo.com
Tamsin	Eades	Illumina	teades@illumina.com
Ralf	Moser	Illumina Australia Pty Ltd	rmoser@illumina.com
Carl	Kirkwood	Bill and Melinda Gates Foundation	carl.kirkwood@gatesfoundation.org
Adam	Winterhalter	Sysmex Australia Pty Ltd	winterhalter.adam@sysmex.com.au
Michelle	Forrester	Sysmex Australia Pty LTD	forrester.michelle@sysmex.com.au
Vincent	Clarke	Sysmex Australia Pty Ltd	clarke.vincent@sysmex.com.au
Garrick	Wilson	Sysmex Australia Pty Ltd	wilson.garrick@sysmex.com.au
Tina	Howe	Carl Zeiss Pty. Ltd.	tina.howe@zeiss.com
Sherrie	Perez	Genetic Signatures	sherrie@geneticsignatures.com
Jackson	Jones	Genetic Signatures	jackson@geneticsignatures.com
Diane	Rowling	Metro North Hospitals and Health Service	diane.rowling@health.qld.gov.au
Mark	Schembri	University of Queensland	m.schembri@uq.edu.au
Laura	Arrowsmith-Hudson	PerkinElmer	laura.arrowsmith-hudson@perkinelmer.com
Barbara	Drigo	Future Industries Institute, University of South Australia	barbara.drigo@unisa.edu.au
Terry	Scanlon	Bruker Pty Ltd	terence.scanlon@bruker.com
Thien	Tran	Bruker Pty ltd	thien.tran@bruker.com
Jon	Iredell	University of Sydney	jonathan.iredell@sydney.edu.au
Lara	Herrero	Griffith University	l.herrero@griffith.edu.au
Ian	Watson	WA Department Of education	ianteach@iinet.net.au
Rebecca	LeBard	University of New South Wales	r.lebard@unsw.edu.au
Scott	Beatson	University of Queensland	scott.beatson@uq.edu.au
David	Gordon	Australian National University	david.gordon@anu.edu.au

Notes

Notes

Notes

Notes

asm2019 SOUTH AUSTRALIA

30 June - 3 July

Adelaide Convention Centre

www.theasm.org.au

The Australian Society for Microbiology

bringing Microbiologists together

Microbiology & Humanitarian and Development Solutions

Rubbo Oration Speaker:

Prof Tilman Ruff

University of Melbourne, AUS

Invited Plenary Speakers:

Dr Marnie L Peterson

University of Wyoming, USA

A/Prof Noah Fierer

University of Colorado, USA

Great Scientific & Social Program already in place